Measurements and models of electrically-evoked motion in the gerbil organ of Corti

by

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B.S. Biomedical Engineering, Boston University, 1992
M.S. Biomedical Engineering, Boston University, 1995

Submitted to the Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology in partial fulfillment of the requirements for the degree of

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ABSTRACT

A cell with extraordinary motile ability exists in our inner ear, the outer hair cell. Outer hair cell (OHC) motility can occur at acoustic frequencies and play a key role in mammalian cochlear frequency selectivity and hearing sensitivity. To date, the mechanism of cochlear amplification is not well understood and remains a matter of controversy.

In order to understand the role of OHC motility in cochlear micromechanics we developed a technique to measure the mechanical responses within the organ of Corti (OC) due to OHC forces. We used an excised cochlea preparation because it provided a good view of the organ and allowed us to compare the resulting responses of hundreds of cells simultaneously. The tissue was stimulated electrically using sinusoidal current, and the resulting motion was captured at specific phases within the stimulus period using stroboscopic video microscopy. Animations of this motion were created, and the displacement magnitude and phase for each structure were calculated using two dimensional cross-correlation. With these techniques we were able to detect displacements as low as ten nanometers.

The frequency responses of electrically-evoked vibrations from the apical and middle turn had low pass filtering characteristics with cutoff frequencies near or below the estimated characteristic frequency of the imaging location. Using a simple one dimensional electrical model of our excised cochlea preparation, we hypothesize that the electrical properties of the stria vascularis play an important role in shaping the frequency response of individual structures. The vibration pattern of the organ was complex and changed with frequency. These changes suggest that at least two OC vibration modes are excited by OHC motility. At all frequencies OHC motility induced oscillatory fluid flow in the tunnel of Corti. We modeled the tunnel of Corti as an elastic tube and showed that it can support a traveling wave. The tunnel of Corti wave could travel without significant attenuation for distances larger than the wavelength of the cochlear traveling wave at its peak.

The classical view of cochlear partition vibration is that the structure simply bends in phase along the radial dimension, and that there is no coupling between adjacent sections other than that provided by the fluid above and below the OC. Our findings challenge the classical view of cochlear partition vibration, and support the existence of multiple vibration modes. In addition, the presence of fluid flow in the tunnel of Corti in response to OHC contractions suggests that a second traveling wave provides longitudinal coupling between adjacent sections. Such coupling may be critical for cochlear amplification.

Thesis Supervisor: David C. Mountain
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I have had a great journey, I have so many I wish to thank. My advisor Professor David Mountain – when my family and friends ask me to describe him I say “he talks about science and his eyes pop out from excitement” – honestly it must be a completely involuntary response! This excitement has kept me going through the many rough times of this thesis when experiments were impossible to complete and data hard to comprehend. Professor Mountain, I want to thank you for being there not only as a scientific advisor but as a mentor and friend every step of the way.

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I will never forget the last few minutes of my thesis defense, May 10th, 2002 about 3pm. So many thoughts in my head and nothing coming out of my mouth. Standing between a projection screen with the pictures of my family and me, the pictures of my husband and me … and on the other side a wonderful crowd, friends, classmates, professors, in a few minutes the moment would be gone, in a few minutes all the thoughts in my head would be just mine – for the moment was overwhelming. Let me share these thoughts with you. I intended words of gratitude. For my father Marco and my mother Fotini, my angels on earth, loving and protecting me every step of the way, guiding me and
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Look at the time, the moment is over --- thank you for listening.
To my husband, Kostantinos Zafiriou
and to my parents, Marcos and Fotini Karavitaki
ITHAKA (1911)

Constantine P. Cavafy

When you set out for Ithaka
ask that your way be long,
full of adventure, full of instruction.
The Laistrygonians and the Cyclops,
angry Poseidon—do not fear them:
such as these you will never find
as long as your thought is lofty, as long as a rare
emotion touch your spirit and your body.
The Laistrygonians and the Cyclops,
angry Poseidon—you will not meet them
unless you carry them in your soul,
unless your soul raise them up before you.

Ask that your way be long.
At many a summer dawn to enter
—with what gratitude, what joy—
ports seen for the first time;
to stop at Phoenician trading centres,
and to buy good merchandise,
mother of pearl and coral, amber and ebony,
and sensuous perfumes of every kind,
sensuous perfumes as lavishly as you can;
to visit many Egyptian cities,
to gather stores of knowledge from the learned.

Have Ithaka always in your mind.
Your arrival there is what you are destined for.
But do not in the least hurry the journey.
Better that it last for years,
so that when you reach the island you are old,
rich with all you have gained on the way,
not expecting Ithaka to give you wealth.
Ithaka gave you the splendid journey.
Without her you would not have set out.
She hasn’t anything else to give you.

And if you find her poor, Ithaka has not deceived you.
So wise have you become, of such experience,
that already you will have understood what these Ithakas mean.

Translation source: Six Poets of Modern Greece, edited by E. Keeley & Ph. Sherrard
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Chapter 1

Introduction

A. General purpose

Von Békésy's work has had a major impact on our understanding of cochlear mechanics (von Békésy, 1960). Von Békésy's observation of the basilar membrane (BM) traveling wave offered a connection between the anatomical structure of the cochlea and its function as a mechanical frequency analyzer. Energy introduced in the system propagates from the basal to the apical part of the cochlear partition. At some point along the length of the partition, depending on the stimulus frequency, the impedance becomes very small and the motion of the partition peaks. Beyond this point, the amplitude of the motion decreases due to frictional and inertial forces. Von Békésy found this wave to be linear, and present at high sound pressure levels.

For the past two decades many differences have been noted between von Békésy's measurements on excised cadaver cochleas and those on live animals or in vitro preparations where the condition of the organ is carefully monitored and controlled to resemble in vivo conditions. Indeed, the traveling wave exists and high vibration amplitudes can be evoked by much lower sound pressure levels than von Békésy used. In addition, the traveling wave at the region of its best frequency exhibits a nonlinear dependence on stimulus level and is sharply tuned. It is these differences between the data obtained from a cadaver cochlea and those obtained from a living cochlea that gave rise to such terms as “passive” vs. “active” traveling wave and has led many scientists to search for the “active” element responsible for injecting energy into the traveling wave as this passes through the active region near the best frequency.
This “active” element is currently thought to be located within the outer hair cells (OHCs). The OHCs cells are located in the organ of Corti and have been found to alter their length in response to changes in their membrane potential, a property usually referred to as “electromotility”. OHC contractions near the resonant place have been hypothesized to be out of phase with the frictional forces that oppose the motion of the BM. Consequently, the OHC would introduce negative damping and increase the displacement of the BM.

Most of our knowledge on OHC function comes from isolated OHC studies. To date, the in vivo action of the OHCs in cochlear amplification is poorly understood and remains a matter of controversy. The goal of this thesis was to put the OHC back into the cochlea and measure the electrically-evoked motion of the OHCs and its effect on the micromechanical motion of the organ of Corti. Understanding the motion of the organ of Corti is of great importance, since this motion shapes the motion of the inner hair cell hairbundles which in turn is transduced and passed on to the brain via the auditory nerve fibers.

B. Thesis Organization

Each chapter in this thesis has been written and formatted as a paper for publication in specific journals. Thus the format is not consistent among all chapters and references are provided at the end of the chapter in which they occur. There is some overlap of material in the methods section of each chapter.

In chapter 2 we provide a detailed description of our general methodology. The results of this chapter present the first systematic study on the internal micromechanical motion of the organ of Corti, due to electrically-evoked OHC contractions at low frequencies. We show that the motion of the OHCs is a combination of length change and pivoting and that the estimated percent length change is similar to that measured in isolated OHCs.

In chapter 3 we demonstrate that the motion of the organ of Corti is complex and the relative amplitude and phase of motion of individual structures changes with frequency. This frequency dependent change of the relative motion of individual structures suggests that the organ of Corti has multiple modes of vibration.
In chapter 4 we explore the spatial and frequency variation of the OHC input and OHC output in the electrically-stimulated excised cochlea preparation. In addition, we use a simple electroanatomical model of our preparation to estimate intracochlear and intracellular potentials and relate our electrical stimuli to those used in isolated OHCs.

In chapter 5 we demonstrate that OHC motility induces oscillatory fluid flow in the tunnel of Corti at all frequencies. Using a one-dimensional elastic tube model of the tunnel of Corti we show that the tunnel can support a traveling wave and that this wave can travel without significant attenuation for distances larger than the wavelength of the basilar membrane traveling wave at its peak.

C. Implications on cochlear micromechanics

The classical view of cochlea partition vibration is that the structure simply bends. This means that if you cut a radial section along the cochlear partition and look at its profile you would always see it moving in phase from end to end and either going up or down. In addition, a common assumption of classical cochlear models is that the cochlear partition acts as a point stiffness and that longitudinal coupling in the cochlear partition is provided by the fluid above and below the organ of Corti.

Our findings challenge the classical view of cochlear partition vibration, and support the existence of multiple vibration modes. Our conceptual framework is that the vibration of the organ of Corti is the superposition of two components, the pressure-driven component and the OHC-driven component. Depending on the type and level of excitation the relative contribution of these components will change. Our methodology emphasizes the OHC-driven component. Our results show that when the OHC-driven component dominates the relative motion of OHCs can change with frequency suggesting that the cochlear partition has multiple vibration modes. In addition, the presence of fluid flow in the tunnel of Corti in response to OHC contractions suggests that a second traveling wave provides longitudinal coupling between adjacent longitudinal sections. Such coupling may be critical for cochlear amplification.
D. References

Chapter 2

Electrically-evoked micromechanical motion in the excised gerbil cochlea at low frequencies

ABBREVIATIONS

AE, artificial endolymph; AP, artificial perilymph; AZ, arcuate zone; BM, basilar membrane; CC, Claudius's cell; CF, characteristic frequency; HC, Hensen's cell; IHC, inner hair cell; IPC, inner pillar cell; OC, organ of Corti; OHC, outer hair cell; OHC1, first row of outer hair cell; OHC2, second row of outer hair cell; OHC3, third row of outer hair cell; OPC, outer pillar cell; PC, pillar cell; PZ, pectinate zone RL, reticular lamina; RM, Reissner's membrane; SM, scala media; ST, scala tympani; SV, scala vestibuli; TM, tectorial membrane; TM_edge, edge of the tectorial membrane

I. INTRODUCTION

Gold in 1948 was the first to hypothesize that cochlear tuning and sensitivity was the result of a feedback system consisting of a forward and a reverse transduction process. Due to this feedback process, in phase correspondence between the feedback force and the
cochlear partition velocity would lead to an enhancement of the cochlear tuning by canceling the viscous forces. It is currently hypothesized that this feedback is provided by voltage-dependent outer hair cell (OHC) length changes.

Evidence for the OHC direct involvement in cochlear function came with the observation that by eliminating the OHCs and leaving the rest of the organ of Corti (OC) intact leads to a significant loss of sensitivity (Evans and Harrison, 1976; Dallos et al., 1978; Liberman and Kiang, 1978). Stimulation of cochlear efferents, which terminate mostly on OHCs, altered the production of distortion-product emissions (Mountain, 1980) and tuning of inner hair cells (IHC) (Brown et al., 1983). The discovery of electrically-evoked otoacoustic emissions (Mountain et al., 1980; Hubbard and Mountain, 1983) and of voltage dependent length changes in isolated OHCs (Brownell et al., 1985; Kachar et al., 1986) provided more evidence for the active role of the OHCs in cochlear function.

There is currently little understanding on how OHC length changes affect the vibration characteristics of the cochlear partition. The intricate anatomy of the OC and the different mechanical properties of the cellular components (Olson and Mountain, 1991, 1994; Naidu and Mountain, 1998), suggest that the vibration pattern will be complex. Moreover, both the anatomy and the mechanical properties of individual cells in the OC change along the length of the cochlea. Therefore, in order to understand the motion of the OC, measurements have to be made at multiple positions within the organ and multiple locations along the cochlea, for multiple stimulus conditions.

To investigate the OC mechanics most researchers have made measurements along the basilar membrane (BM) or along the reticular lamina (RL). A comprehensive review is given by Ulfendahl (1997) and Robles and Ruggero (2001). Little information is available on the internal micromechanical motion of the OC due to OHC contractions. Measurement of this internal motion has been a challenge because it is not optically accessible. We developed an excised cochlea preparation (modification of Ulfendahl et al., 1989a, b) which allowed us to image the responses of hundreds of cells simultaneously at multiple focal levels, starting from the tectorial membrane (TM) level down to the BM level, at 2 μm intervals. We present data from the apical and middle turn. The characteristic frequency (CF) of our measurement locations was estimated using the place-frequency map of the gerbil cochlea (Muller, 1996) and ranged from 0.4 to 4 kHz. We show that in response to electrically-evoked OHC
contraction, the motion of the OC at low frequencies is complex, and is qualitatively similar in both the apical and the middle turn of the gerbil cochlea.

II. METHODS

A. Surgical preparation of gerbil cochleae

Young female Mongolian gerbils were decapitated after being deeply anaesthetized with an intraperitoneal injection of sodium pentobarbital (60mg/Kg) or using a mixture of ketamine (0.16 mg/g) and xylazine (0.008mg/g). The procedures followed an institutionally approved protocol with guidelines provided by the Laboratory Animal Care Facility at Boston University. Following decapitation both temporal bones were excised and muscular and brain tissue was removed. After opening the bulla the bones were immersed in oxygenated culture medium (Leibovitz L-15) supplemented with 5 mM D-Glc. The pH of the solution was adjusted to 7.3 at the beginning of each experiment using NaHCO₃. In later experiments, the medium was a Cl⁻ modified perilymph-like solution composed of 140 mM D-GlcA, 6.6 mM NaCl, 100 μM CaCl₂, 3 mM KCl, 5 mM NaH₂PO₄, 100 μM MgCl₂, 5 mM D-Glc, 5 mM Hepes (298 mOsm, pH 7.3 adjusted using 1M NaOH). Both solutions were at room temperature (~18°C) during the experiment. The later solution was formulated to improve the condition of the preparation and the viability of the cells. Using this solution we were able to collect data for a maximum of nine hours following decapitation.

The next step in the surgical procedure was to remove the tympanic membrane, the malleus, incus and parts of the semicircular canals. Enough temporal bone was left attached to provide a stable means of holding the cochlea. The next step was to expose the turn of interest without damaging Reissner's membrane (RM), and remove as much of the bone surrounding the scala tympani (ST) of the lower turn so that a better optical path could be established.

For experiments in the apical (low frequency end) turn, the round window membrane was removed along with most of the bone surrounding the basal (high frequency end) turn. This allowed access to the modiolus between the basal and middle turns. The
modiolus was then cut with a pair of forceps and the basal turn was removed along with the
bone of the ST and sometimes the scala media (SM) of the middle turn. Next, a hole was
made with a sharp pick at the very tip of the apical turn. Using the band of stria vascularis as
a guide, the bone covering the scala vestibuli (SV) was removed using a thin pair of forceps,
and the apical turn was exposed. This procedure ensured that the scala media remained
intact.

For experiments in the middle turn, the basal turn of the cochlea was removed and a
hole was made in the apical turn as before. Using a thin pair of forceps, the apical turn was
completely removed and the bone covering the scala vestibuli of the middle turn was
carefully peeled away. As before, this procedure ensured that the scala media was
anatomically preserved (Figure 2-1). The average time for the surgical procedure was about
20 minutes following decapitation.

B. Video microscopy system

Following dissection, the preparation was mounted in a custom made chamber with
a cover glass bottom and then placed on the stage of an upright microscope (Olympus,
BX50WT). The microscope was sitting on a vibration-isolation table with a second degree of
isolation provided by a steel slab supported on tennis balls. In later experiments, the cochlea
holder was modified to allow manual rotation in three dimensions, which permitted control
over the viewing angle of the organ. A 4x (Olympus 4x, 0.13NA) lens was used for orienting
the cochlea and later positioning the electrodes (described in the next section). Figure 2-2
shows the view of the cochlea using the 4x objective for a middle turn experiment. A 20x
(Olympus 20x, 0.5 NA) or a 60x (Olympus 60x, 0.9 NA) water immersion lens with an
additional 2x magnification was used for detailed observation of the OC in the regions of
interest. The objective was connected to a nanopositioner (PI, PIFOC P-723) equipped with
a piezoelectric driver. The PIFOC driver was controlled remotely via a programmable
amplifier module (PI, E-662), and allowed precise control over the focal level. Figure 2-3
shows the view of the cochlea at different focal levels using the 20x objective. The resolution
of the images using this objective was 432 nm/pixel.
Figure 2-1: Midmodiolar cross section of a gerbil cochlea (slide provided by J. C. Adams) to illustrate our middle turn preparation. The apical and basal turns have been removed and a small opening has been made in the scala vestibuli above the region of interest. Note that Reissner’s membrane is intact and therefore the anatomical architecture of the entire turn is preserved. Also shown is the relative electrode placement for the electrical stimulation paradigm.

Figure 2-2: Low magnification surface view of our excised cochlea preparation. This is a middle turn preparation of a left cochlea and it spirals clockwise towards the apex. Notice that the entire turn of interest is present. The arrow points to the opening of scala vestibuli above the region of interest. The opening is outlined for clarity.
Figure 2-3: High magnification surface views of our excised cochlea preparation. All views are from the same cochlea location and were acquired by focusing the objective at different levels across the OC. Panel A: Tectorial membrane (TM) level, the arrows point to the TM radial fibers and the edge of the TM (TM_edge). Panel B: cuticular plate (CP) level, arrows point to the IHC hairbundles (IHC_s) and the OHC1 hairbundles (OHC1_s). Panel C: OHC basal end, arrows point to the outer pillar cells (OPC), first row of OHCs (OHC1), second row of OHCs (OHC2), third row of OHCs (OHC3) and the Hensen’s cells (HC). Panel D: Basilar membrane (BM) level, the arrows point to the arcuate zone of the BM (BM_AZ), the radial fibers of the pectinate zone of the BM (BM_PZ) and the edge of the HC (HC_edge).
A CCD (Hamamatsu, C2400-77) camera was mounted on the phototube of the microscope. Analog contrast enhancement and brightness enhancement was accomplished by using an image processor (Hamamatsu, Argus-20). The output of the image processor was connected to an externally triggered frame grabber (Scion Corporation, AG-5) for real time frame capture and averaging.

C. Electrical stimulation: hardware and software

AC current was delivered through glass pipettes filled with 3M NaCl. The input current electrode was placed in the SV of the same turn that responses were measured. The return current electrode was placed near the former location of SV in the next more basal turn (Figure 2-1). The pipettes were sealed with agar at their tip to prevent NaCl leakage and were connected to an optically isolated constant current source. The inner diameter of the pipettes ranged from 100 to 300 μm. The injected current was monitored by measuring the voltage across a 100 Ω resistor in the current return path. During electrical stimulation, current levels were limited to 4 mA or less to prevent tissue damage. The voltage drop across the fluid between the electrodes was about 150mV/mA. A computer controlled, Tucker-Davis Technologies (TDT), System II analog interface was used to generate the input to the current source and to store the stimulating current waveform.

The stimuli were sine waves with frequencies from 30 Hz to 120 Hz. Movements synchronized to the stimulus frequency were captured using stroboscopic illumination. A custom made current source was used to deliver current pulses of 200 mA peak to a light emitting diode (LED, model AND190AYP) emitting more than 50 Cd at a 4° viewing angle. The LED was mounted on a holder designed to replace the light source of the microscope. The input pulses to the strobe system were generated using the TDT system. The pulses occurred at fixed phases within the period of the stimulus with duration equal to 10% of the stimulus period.

Data were collected for eight equally spaced, randomized phases and for two conditions: (1) with the stimulating current being present and (2) with the stimulating current turned off, referred to as the “no-stimulus condition”. The no-stimulus condition gave us an
estimate of the magnitude of the minimum resolvable motion of our system and also verified that the motions observed were due to the current being present. For each stimulus period, pulses occurred only at one particular phase. Thus, to collect data from eight phases the same frequency was played eight times. For each frequency/phase combination the stimulus was on for 1 minute to provide enough images for subsequent averaging. Typically, we used a 16-frame average. The video frames of interest were subsequently digitized and animations of the observed motion were created by playing the images from each phase in succession.

D. Image processing and motion estimation

Electrically-evoked motion was estimated using two-dimensional (2D) cross-correlation. In traditional signal processing cross-correlation is performed to obtain a measure of similarity between two signals $f_1(t)$ and $f_2(t)$ as a function of a scanning parameter $\tau$. This parameter is usually the time shift of one signal with respect to the other. An important application of cross correlation in image processing is in the area of template matching, where the goal is to find the closest match between an unknown image and a set of known images (Gonzales and Woods, 1993). The scanning parameter in this case is the spatial shift of one image with respect to the other. Since we are interested in 2D discrete images the spatial shift applies for any combination of $x$ (radial dimension) and $y$ (longitudinal dimension) on the image plane. For the discrete 2D case the cross correlation of two images $f(x,y)$ and $g(x,y)$ is given by

$$R_{f,g}(x, y) = \frac{1}{MN} \left( \sum_{m=0}^{M-1} \sum_{n=0}^{N-1} f^*(m,n) g(x + m, y + n) \right)$$  \hspace{1cm} (2.1)

for $x = 0,1,2,\ldots, M-1$ and $y = 0,1,2,\ldots, N-1$. The symbols $M, N$ refer to the image's radial and longitudinal dimensions, respectively. The following cross correlation theorem

$$R_{f,g}(x, y) \leftrightarrow F^*(u,v)G(u,v)$$  \hspace{1cm} (2.2)
holds for \( u=0, 1, 2, \ldots, M-1 \) and \( v = 0, 1, 2, \ldots, N-1 \). The functions \( F, G \) are the frequency domain representations of \( f, g \). The theorem states that the cross correlation of two images in the frequency domain is the result of a simple multiplication of their Fourier transforms. The * indicates that the complex conjugate of the function is taken. Cross-correlation functions computed with fast Fourier transforms (FFTs) are often referred to as circular cross-correlation functions, since the FFT treats the image as though it represents one repeat of a periodic pattern. As a result the computed cross-correlation is periodic. Since the motions in our experiments were small compared to the extraction size, the largest peak near the origin was chosen for the displacement estimate. If the displacement was larger than half the image size then the peak was wrapped around to the origin and the phase adjusted accordingly.

Cross-correlation was used to estimate radial and longitudinal displacements and it is demonstrated in Figure 2-4 for the one-dimensional case. In this method two images taken at different conditions are considered at a time. In our case, one of those images is the one taken at the no-stimulus condition of a particular phase and is considered as the reference image. The other image is the one taken at the phase corresponding to the no-stimulus condition. Cross correlation between the two images was performed by first extracting a portion of the image containing a feature of interest like the edge of a hair cell (Figure 2-4A). The same portion was extracted from an image taken at a different stimulus phase. (Figure 2-4B, C, D).

It is important to note that the extractions of interest were chosen to have a well defined edge and thus their resulting intensity profiles were single-peaked (Figure 2-4E, F, G, solid square markers). Single-peaked profiles had the advantage of giving a well defined cross-correlation peak. Also, in Figure 2-4E, F, G notice that the beginning and the end of the intensity profiles do not have the same value. We found that this caused errors in the estimation of motion. To solve this problem, and accurately extract the peak of the intensity profile, the images were high pass filtered (not shown in Figure 2-4) using the following kernels

\[
S_x = \begin{bmatrix} 1 & 0 & -1 \\ 2 & 0 & -2 \\ 1 & 0 & -1 \end{bmatrix}
\] (2.3)
Figure 2-4:

Panel A: This is a high magnification surface view of the organ of Corti captured with the described video stroboscopy system at one particular phase of the stimulus (exp. 131). Several structures of interest are shown: the basal end of IHCs, the head of the pillar cells (PC), the three rows of OHCs and the area of the HC. In all our images, the radial dimension points from spiral lamina to spiral ligament and the longitudinal dimension from base to apex. Radial motions towards the spiral ligament and longitudinal motions towards the base are positive by convention. To estimate these motions a portion of the image containing a feature of interest like the edge of a hair cell was extracted from each of the eight phases and the corresponding no-stimulus conditions. For simplicity we present the analysis for three time points.

Panels B,C,D: Shown are three time frames of the edge of an OHC. Each panel is a matrix of 16x16 pixels (432 nm resolution). For illustration purposes the analysis is shown for one row of pixels (indicated in each time frame), but during motion estimation the analysis is done for the entire extracted image.

Panels E,F,G: The intensity of each pixel within the selected row is plotted as a function of radial position (filled squares). Note that the shape of the intensity profile could be similar (but not identical) from one time frame to the next but shifted with respect to the radial position. The next step was to quantify this shift. Because the motion of interest was usually less than a pixel, interpolation was used (filled circles between successive squares). The resulting images have a resolution of 27nm/pixel.

Panels H,J: Cross correlation computed with fast Fourier transforms was used to quantify the shift that yields the best fit between two images. For the curves shown in panels E and F the best fit was at a shift of 320 nm towards the left and for the curves in panels E and G the best fit was at a shift of 260 nm towards the right.

Panel J: Cross-correlation was repeated for all the stimulus phases to derive the time waveform of the displacement shown by the squares. Fourier analysis was then performed on the time waveform and from the fundamental component (F1) we estimated the peak amplitude and phase of motion for each stimulus frequency.
where $S_x$ refers to the kernel applied in the radial direction, and $S_y$ to the kernel applied in the longitudinal direction. These kernels are also known as Sobel operators (Gonzales and Woods, 1992) and were chosen over other gradient operators due to their advantage of detecting edges while also having a smoothing effect, therefore reducing additional noise introduced by high pass filtering the images. Both kernels operated on a pixel-by-pixel basis.

Because the motion of interest was usually less than our pixel size, the next step was to interpolate the extracted images. For simplicity we outline our methodology for the one dimensional case (for example the radial dimension). A detailed analysis of this procedure is given in Oppenheim and Schafer (1989). Our objective was to up-sample the image $I[m]$ to $I_{i}[m]$ as given by

$$I_{i}[m] = I\left[\frac{m}{L}\right], m = 0, L, 2L, \ldots, (M-1)L$$

(2.5)

where, $I_{i}[m]$ is the interpolated version of $I[m]$, $L$ is the interpolation factor and $M$ is the radial length of the image. One way to derive $I_{i}[m]$ is by first using an expander on $I[m]$ such that

$$I_{e}[m] = \sum_{k=0}^{k=(M-1)} I[k] \delta[m - kL]$$

(2.6)

where $I_{e}[m]$ is the expanded version of $I[m]$ and $\delta$ is the unit impulse function. $I_{e}[m]$ is then obtained by lowpass filtering $I_{e}[m]$ with a cutoff frequency of $\pi/L$ and gain equal to $L$. We implemented this procedure in the spatial-frequency domain. Notice that the Fourier transform of $I[m]$ and $I_{e}[m]$ is given by
\[
F\{I[m]\} = \sum_{k=0}^{k=(M-1)} I[k]e^{-j2\pi mk} = \tilde{I}(e^{j2\pi m})
\]

\[
F\{I_e[m]\} = \sum_{k=0}^{k=(M-1)} I[k]e^{-j2\pi mk} = \tilde{I}(e^{j2\pi m}).
\]  

(2.7)

From equation 2.7 we see that \(\tilde{I}(e^{j2\pi m})\) is a frequency-scaled version of \(\tilde{I}(e^{j2\pi m})\). We therefore expanded the Fourier transform of our extracted image in the spatial frequency domain by appending zeros to expand it to the desired interpolation size. We then calculated the inverse Fourier transform of the image to obtain the interpolated version of our original image. Notice that in our analysis our images are band-limited therefore the form of the low pass filter is rectangular. Such a filter is ideal since the amount of distortion in the added samples is zero. We used a 16x interpolation factor and with that our final pixel resolution was 27 nm. The resulting interpolated intensity profiles for the images shown in Figure 2.4 B, C, D are shown in Figure 2.4 E, F, G (filled circles between successive squares, here the interpolation factor is limited to 4x for visualization purposes).

Once the extracted images were interpolated, the cross correlation between two images was computed using fast Fourier transforms. The location of the cross correlation peak, with respect to the origin, gave us an estimate of the magnitude and direction of motion between the original images (Figure 2.4 H, I). This procedure was then repeated for all the stimulus phases to derive the time waveform of the motion (Figure 2.4 J).

Fourier analysis was then performed on the time series to estimate the peak magnitude and the phase of motion for each frequency. Specifically we used the complex exponential Fourier series (Kamen, 1987), to reconstruct the time waveform, as given by

\[
x(kT) = \sum_{n=0}^{N-1} a_n e^{j2\pi nk/N}, k = 0, \pm1, \pm2, \ldots
\]  

(2.8)

where \(N\) is the number of samples per period, \(n\) is the harmonic number, \(k\) is the discrete increment of time, i.e. \(t = kT\), where \(T\) is the sampling interval, and finally

\[
a_n = \frac{1}{N} \sum_{k=0}^{N-1} x(kT)e^{-j2\pi nk/N}, n = 0, 1, \ldots, N - 1.
\]  

(2.9)
We then computed the magnitude and the phase of motion of the function \((n = 1)\) by

\[
|x(kT)| = \sqrt{\text{Re}^2(a_n) + \text{Im}^2(a_n)}
\]

\[
\angle x(kT) = \tan^{-1}\frac{-\text{Im}(a_n)}{\text{Re}(a_n)}
\]

Because conventional Fourier analysis references phase to a cosine, we added 90° to our motion phase data to reference them to our sine phase stimuli. From this 2D analysis we were able to estimate motion in two directions. The first was the radial direction (referring to the axis running from spiral lamina to spiral ligament) and the second was the longitudinal direction (referring to the axis running along the organ of Corti from base to apex). By convention positive displacements are towards the spiral ligament in the radial direction, and towards the base in the longitudinal direction (Figure 2-4A).

There are several implicit assumptions in our method for motion estimation: (1) the motion is much smaller than the analysis window (16x16 pixels interpolated to 256x256 pixels); (2) the motion is sinusoidal with frequency equal to the stimulus frequency; and (3) the light transmitted through any particular element or through a pixel, does not change when the tissue is electrically stimulated or moves.

E. Control experiments

1. Electrical stimulation

At the beginning of each experiment, after positioning the electrodes, a test stimulus was presented at a frequency of 120 Hz and with the strobe flashing at 126 Hz. Under these conditions a 6 Hz motion was observed if the electrodes were positioned correctly and the preparation was in good condition. If no motion was observed, the electrodes were repositioned and the test stimulus played again. If motion was observed, then the level of the current was reduced to the point where motion could just be detected visually. This was done to avoid over-stimulation of the tissue.
2. Motion artifacts

During data collection, low frequency building vibrations and drift in the cochlear holder introduced mechanical noise in our measurements. This type of noise was reduced to a large extent by collecting a no-stimulus condition before each stimulus phase. Cross correlation was then performed between the image at a particular phase and the image at the corresponding no-stimulus condition. This control also verified that the motions observed were due to the current stimulus.

3. Frame grabber and image processing algorithm

The following experiment was designed to test if the frame grabber captured the correct frames and if the motion estimation algorithm gave accurate results. A piezoelectric probe (Olson and Mountain, 1991) was used to produce a known sinusoidal displacement magnitude for frequencies ranging from 30 Hz to 3 kHz. Images of the force probe were collected using our stroboscopic system. The displacement of the probe was then estimated from these images using our cross correlation technique. The displacement of the probe was also measured using a motion transducer system (Opto Acoustic Sensors, Inc., Angstrom Resolver Model 201), and the results were compared with the displacements obtained using our motion estimation algorithm. The magnitude results were within 1.5 dB and the phase results were within 15 degrees for the frequencies between 30 to about 2 kHz. Beyond this frequency the response of the force probe was very large and our algorithm tended to underestimate the motion.

F. Anatomical orientation

Typically, at the end of each experiment, images were collected at multiple focal levels to identify the anatomical orientation of our excised cochlea preparation with respect to our imaging plane (Lp). This orientation was important to know when interpreting the results of our motion estimation analysis for reasons that will become explicit in the following sections. In Figure 2-5 we show cartoons of the radial and longitudinal view of the
A. Radial View

B. Longitudinal View

Figure 2-5: Anatomical cartoons showing the radial and longitudinal views of the OC. Also, shown are possible orientations of our imaging plane ($I_p$) to illustrate the different angles that are important for understanding the motion estimation results from our surface views. $\theta_r$, $\theta_L$: angle between the long axis of the OHCs and the RL, in the radial and longitudinal direction respectively; $\beta_r$, $\beta_L$: angle between the $I_p$ and RL; $\gamma_r$, $\gamma_L$: angle between the $I_p$ and the long axis of the OHCs.
OC and identify the angles of interest that will be used in the analysis of our results. The long axis of the OHC is oriented at an oblique angle with respect to the RL in both the radial direction \( (\theta_R) \) and the longitudinal direction \( (\theta_L) \). Because the \( I_p \) is not always parallel to the RL an additional angle is introduced between the \( I_p \) and the RL \( (\beta_R, \beta_L) \) for the radial direction, \( \beta_L \) for the longitudinal direction) and between the \( I_p \) and the long axis of the OHC \( (\gamma_R, \gamma_L) \) for the radial direction, \( \gamma_L \) for the longitudinal direction). By convention, when the spiral ligament or apical end of the \( I_p \) is below the RL (as shown in Figure 2-5) then the angle \( \beta \) is negative. The relationship between the angles is

\[ \theta = \gamma - \beta. \]  

(2.11)

Using images taken at multiple levels we were able to estimate \( \gamma_R, \gamma_L, \beta_R, \beta_L \), and use these angles to calculate \( \theta_R, \theta_L \). In order to estimate \( \gamma_R \) and \( \gamma_L \) we extracted the same region of interest (ROI) from all the levels. We used our two dimensional cross correlation technique to estimate the radial and longitudinal displacement of the edge of the cell, from one level to the next. We repeated this analysis for multiple ROIs and from all three rows of OHCs, and plotted the computed displacements as a function of level (vertical dimension). We then computed the slope between successive points in all curves by dividing the incremental change in the radial \( (\Delta R) \) and the longitudinal dimension \( (\Delta L) \) by the incremental change in the vertical dimension \( (\Delta H) \). The angle of interest, \( \gamma_R, \gamma_L \) between successive points was then computed by

\[ \gamma_R = \tan^{-1}\left(\frac{\Delta H}{\Delta R}\right) \]  

(2.12)

and

\[ \gamma_L = \tan^{-1}\left(\frac{\Delta H}{\Delta L}\right). \]  

(2.13)

A histogram of all the angles was plotted and the mode of the histograms was chosen as our estimate of \( \gamma_R, \gamma_L \).
In order to estimate $\beta_R$, $\beta_L$, we used our multiple level images and identified those levels where key structures such as the hairbundles of the IHCs and OHCs came first in focus. We assumed that when the $I_p$ is parallel to the RL in the radial dimension, we would see the IHCs and OHCs hairbundles in focus at the same time. Similarly, when the $I_p$ is parallel to the RL in the longitudinal dimension, we would see the entire row of a given structure in our field of view (i.e. the entire row of IHC hairbundles) in focus at the same time. For example, in Figure 2-4A notice that the three rows of OHCs are all in focus at the same time for both the radial and longitudinal directions. This means that the $I_p$ is parallel to the RL in both the radial and longitudinal dimensions. The next step in estimating $\beta_R$, $\beta_L$, was to measure the vertical distance ($H$) between the levels that the identified structures came in focus and the radial ($R$) or longitudinal ($L$) distance between those structures. Using these distances $\beta_R$, $\beta_L$, were calculated by

$$\beta_r = \tan^{-1}\left(\frac{H}{R}\right)$$  \hspace{1cm} (2.14)

and

$$\beta_l = \tan^{-1}\left(\frac{H}{L}\right).$$  \hspace{1cm} (2.15)

G. Interpretation of measurements

The orientation of the $I_p$ with respect to the long axis of the OHCs will affect our estimates of motion, in the radial and the longitudinal direction. To illustrate this consider the OHC geometry as shown in Figure 2-6. Here, the OHC is treated as a cylinder in two states: (1) at rest (thin line) and (2) at maximum contraction (thick line). The axis running along the long axis of the cell is the vertical dimension and the axis perpendicular to the long axis can be either the radial or the longitudinal dimension (due to the radial symmetry of the cylinder). Assuming that the volume of the cell remains constant, OHC contraction will result in shortening in the vertical dimension and expansion in the radial or longitudinal dimension. This expansion translates into an increase in the radius of the cell, marked as $R_r$.
Figure 2-6: A. Schematic drawing of an OHC's radial cross section. The cell is shown at rest (thin rectangle) and at the maximum contraction (thick rectangle). H: vertical axis, in this case parallel to the long axis of the cell, generally considered to be the axis parallel to the optical axis; r: radial axis; $R_a$: actual radial displacement; $R_o$: observed radial displacement; all other acronyms as defined in figure 2-5. B. Surface view of the OHC at rest (thin ellipse) and at maximum contraction (thick ellipse).
(actual change of radius). If our imaging plane is perpendicular to the long axis of the cell (i.e. $\gamma_r = 90^\circ$) then the observed change in radius $R_o = R_r$. If our imaging plane is as shown in Figure 2-6A, i.e. $\gamma_r >90^\circ$ then $R_o > R_r$. Specifically, $R_o = R_r / \cos(\gamma_r - 90^\circ)$. Similar arguments hold for the longitudinal dimension. In Figure 2-6B we show the surface view of an OHC, at rest (inner circle) and at maximum contraction (outer circle). The orientation is such that $\gamma_r > 90^\circ$ whilst $\gamma_l = 90^\circ$. In this case $L_o = L_r$ but $R_o > R_r$. Therefore, we are overestimating the radial component of motion.

III. RESULTS

A. Effect of current level on OHC displacements

In Figure 2-7 we show the magnitude and the phase of radial displacements, as a function of stimulus level, from two OHCs. The response magnitude of OHCs increased as the stimulus current increased. At low stimulus levels (when the motion of the cells could just be detected visually) the responses increased linearly with current. As the stimulus level increased further the responses saturated and at very high stimulus levels decreased. The phase of the responses remained constant for all stimulus levels. The first row of OHCs (OHC1) moved 180° out of phase with the third row (OHC3). This phase difference was observed in all of our experiments and will be further addressed in the following sections.

In Figure 2-7 we also show the response at selected current levels (circled points in the graphs). These measurements were collected at the end of each experiment to evaluate the effect of time on the responses. Some of these points matched the ones collected earlier in time, others like the one shown for the 1 mA current in panel B showed a decreased response. Usually, as the current level increases, cells start to swell, and after a period of time they stop contracting.

Finally, note that the current levels used are different for the two cells shown in Figure 2-7. In our preparation, the minimum current needed to evoke detectable OHC motion depended on two factors. The first was the exact position of the input current electrode with respect to the OHCs, and the second was the condition of the preparation. If
Figure 2-7: Peak-to-peak magnitude and phase of radial displacements as a function of stimulus current. The stimulus frequency was 120 Hz. Dotted line is of unity slope. Panel A,C: responses from an apical turn OHC1 (ohc1b, exp. 615). Panel B,D: responses from a middle turn OHC3 (ohc3a, exp. 614). Note that the current axis ranges from 1 mA to 10 mA in panels A, C, and from 0.1 mA to 10 mA in panels B, D.
the input electrode was close to the SV of the imaging location and the preparation was in good condition, then the amount of current needed to stimulate the OHCs was small. A preparation was considered to be in good condition when the cells were not swollen and responses of adjacent cells in the same row were in phase. Typically, the stimulus currents ranged from 0.1 to 4 mA, and for that range of currents, displacements up to about 2 μm were in the linear regime.

B. Anatomical measurements

Figures 2-8 and 2-9 illustrate results from two (1 apical and 1 middle turn) of the four (2 apical and 2 middle turn) experiments in which we estimated γ_R, γ_L, β_R, and β_L. The calculation of these angles was described in the methods section. Figure 2-8A, B shows the apparent radial displacement of individual OHC1, second row of OHC (OHC2) and OHC3 as a function of focal level. As we focused towards the BM, all structures were displaced towards the spiral ligament. Figure 2 8C, D show the histograms of γ_R, which correspond to the apical and middle turn experiment of Figure 2-8A, B, respectively. The average γ_R for each turn was taken to be the mode of the distribution shown in the histograms. For the experiments shown, γ_R = 83° in the apical turn and γ_R = 85° in the middle turn. Finally, for these experiments β_R = -9° in the apical turn and β_R = +14° in the middle turn experiment.

Figure 2-9A, B shows the longitudinal displacement of the same structures shown in Figure 2-8A, B. For both the apical and middle turn experiments the structures were displaced towards the apex (which by convention is in the negative longitudinal direction). Figure 2-9C, D shows the histograms of γ_L, which correspond to the apical and middle turn experiment of Figure 2-9A, B, respectively. The average γ_L for each turn was taken to be the mode of all the angles shown in the histograms. For the experiments shown, γ_L = 87° in the apical turn and γ_L = 89° in the middle turn. Finally, for these experiments β_L = -6° for both experiments.

Using the above numerical results for the two experiments we calculated the values for θ_R and θ_L using equation 2.1. The resulting numbers for the apical turn experiment were
Figure 2-8: Measurements to estimate $\gamma_R$ in the apex (exp. 1008) and middle turn (exp. 1011). Panels A, B show the peak radial displacement of individual OHCs from each of the three rows as a function of depth from the RL to BM. The displacements are positive indicating that as we focused lower into the organ the cells shifted towards the spiral ligament. Due to the large longitudinal tilt in this experiment the cells came in focus at different focal levels and hence the extractions do not all start from the same focal level. In general, extractions from the middle turn experiments span fewer levels since the OHCs in this turn are shorter than those from the apical turn. Panels C, D show the histogram of the angles calculated from panels A,B. The calculation was as described in the text.
Figure 2-9: Measurements to estimate $\gamma_L$ in the apex (exp. 1008) and middle turn (exp. 1011). Panels A, B show the peak longitudinal displacement of individual OHCs from each of the three rows as a function of depth from the RL to BM. The displacements are negative indicating that as we focused lower into the organ the cells shifted towards the apex. Due to the large longitudinal tilt in this experiment the cells came in focus at different focal levels and hence the extractions do not all start from the same focal level. In general, extractions from the middle turn experiments span fewer levels since the OHCs in this turn are shorter than those from the apical turn. Panels C, D show the histogram of the angles calculated from panels A,B. The calculation was as described in the text.
\( \theta_r = 92^\circ \) and \( \theta_l = 93^\circ \), and for the middle turn experiment \( \theta_r = 71^\circ \) and \( \theta_l = 95^\circ \). Similar results were seen in the other two experiments we analyzed. Therefore, our anatomical measurements show that the OHCs in the apical and middle turn are oriented approximately perpendicular to the RL in the longitudinal dimensions. The two turns differ in the radial orientation of the OHCs with respect to the RL, with the apical turn OHCs oriented approximately perpendicular to the RL and the middle turn OHCs oriented at about 70° below the RL.

C. Responses from multiple levels at low frequencies

Figure 2-10 illustrates electrically-evoked displacement measurements from multiple levels in one of our five middle turn experiments (CF~4 kHz). To simplify the presentation of these results we show the motion of all the structures encountered as we move the focal plane from the TM level to the BM level, for specific radial locations. We present here four such radial locations near: (1) the outer pillar cells (OPC), (2) the OHC1, (3) the OHC3 and (4) the Hensen’s cells (HC) region. Notice that in each of the panels, data points are missing for some of the levels. Missing data points indicate that the contrast in these regions was too low for motion analysis.

The results shown in Figure 2-10 are typical for all our middle turn experiments. The OC motion generally increased as we moved from the RL level (~0 \( \mu \)m) towards the basal end of the OHCs (~40-60 \( \mu \)m), and then decreased as we moved further towards the BM (~110-140 \( \mu \)m). This observation is most obvious for the OHC1, OHC3 and HC locations. For these locations also note that the radial component of motion was smaller than the longitudinal component. The above results are typical for all of our experiments.

The motions observed at all levels of the OPC region, the TM level of both the OHC1 and OHC3 region, and the BM level of the OHC1, OHC3 and HC regions, were smaller than those observed at the basal end of the OHCs. As an exception, the OPC region showed a small increase in the radial motion close to the basal end of the OHCs (~25 \( \mu \)m). This increase in the radial displacement of the OPC near the basal end of OHCs was often present in our OPC region extractions.
Figure 2-10: Peak-to-peak radial (filled symbols) and longitudinal (open symbols) displacements as a function of depth relative to the RL, for four radial locations. All responses are from the middle turn region (CF ~ 4 kHz), from the same animal (exp. 1026). The stimulus frequency was 60 Hz. For this experiment $\gamma_r = 91^\circ$ and $\gamma_l = 90^\circ$. Panel A, E: magnitude and phase of OPC region displacements, RL was defined as the top of the OPC. Panel B, F: magnitude and phase of OHC1 region displacements, RL was defined as the top of the OHC1 hairbundles. Panel C, G: magnitude and phase of OHC3 region displacement, RL was defined as the top of the OHC3 hairbundles. Panel D, H: magnitude and phase of HC region displacement, RL was defined as the top of OHC3 hairbundles.
During the depolarizing phase of our stimulus most of the structures moved towards the basal end of the cochlea in the longitudinal direction, and towards the spiral ligament in the radial direction. The following structures moved 180° out of phase, with respect to OHC1, in the radial direction: (1) the pectinate zone under OHC1, OHC3, (2) the OHC3 and corresponding apical part of the Deiter’s cell (DC) and (3) the HC region up to about 75 μm below the RL.

We also measured the responses of the edge of the TM (TM_edge), the inner pillar cell (IPC) and the IHC hairbundles. Motion of all these structures was usually at or below the noise floor of our measurements. We sometimes saw motion in the IHC hairbundles and the TM_edge. The phase of the radial motion of the IHC hairbundles was towards the spiral ligament. The phase of the radial motion of the TM_edge was towards the spiral ligament (i.e. in phase with OHC3 and out of phase with the rest of the TM).

Figure 2-11 shows the corresponding data from our one apical turn experiment (CF~0.4 kHz). As with the middle turn data, motion increased as we moved from the RL level (~0 μm) towards the basal end of the OHCs (~40-70 μm), and decreased as we moved further towards the BM (~100 130 μm). This observation is most obvious for the OHC1, OHC3 and HC locations. Unlike the middle turn, the radial component of motion in the apical turn is larger than or similar to the longitudinal component.

The pattern of motion in the apical turn was similar to that observed in the middle turn. Apical turn motion at all levels of the OPC region, the TM level of both the OHC1 and OHC3 region, and the BM level of the OHC1, OHC3 and HC regions was small compared to that at the basal end of the OHCs. The OPC motion was small compared to the basal end of the OHCs for all levels, except towards 70 μm where we show a small increase in the radial dimension. Also, note that the TM displacement of the OHC1 region was estimated at a higher level and it was larger than that of the OHC3 region.

Finally, similar to the middle turn data, depolarizing current caused most of the structures to move towards the basal end of the cochlea in the longitudinal direction, and towards the spiral ligament in the radial direction. The following structures moved 180° out of phase with respect to OHC1 in the radial direction: (1) the pectinate zone under OHC1, OHC3, (2) the OHC3 and corresponding apical part of the DC and (3) the HC region up to about 75 μm below the RL. In the apical turn we did not see the phase shift at the basal end.
Figure 2-11: Peak-to-peak radial (filled symbols) and longitudinal (open symbols) displacements as a function of depth relative to the RL, for four radial locations. All responses are from the apical turn region (CF ~ 0.4 kHz), from the same animal (exp. 325). The stimulus frequency was 30 Hz. For this experiment $\gamma_R = 73^\circ$ and $\gamma_L = 95^\circ$. Panel A,E: magnitude and phase of OPC region displacements, RL was defined as the top of the OPC. Panel B, F: magnitude and phase of OHC1 region displacements, RL was defined as the top of the OHC1 hairbundles. Panel C, G: magnitude and phase of OHC3 region displacement, RL was defined as the top of the OHC3 hairbundles. Panel D, H: magnitude and phase of HC region displacement, RL was defined as the top of OHC3 hairbundles.
of the DC region (see the 75 μm level of Figure 2-10G). This may be due to the fact that we were not able to image as far down into the DC region for the apical turn as we could in the middle turn.

Motion of the IHC hairbundles and IPC was small and usually in the noise of our measurements. When motion was seen in the IHC hairbundles it was in phase with the motion seen in the middle turn, i.e. towards the spiral ligament. Unlike the middle turn, we were not able to image the TM_edge effectively in the apical turn, so no motion measurements were made.

D. Apparent diameter changes

For most of our experiments we were able to analyze motion for either the lamina (referring to the side of the cell towards the spiral lamina) or the ligament (towards the spiral ligament) side of a cell. For example in Figure 2-4A the structure of interest is the lamina side of the cell. We usually picked the side of the cell that had the greatest contrast in order to maximize the signal to noise ratio. For some experiments we were able to analyze both sides of the cell for all three rows of OHCs.

In Figure 2-12 we show results from a middle turn experiment where we were able to estimate the radial displacements from lamina (R_lam) and ligament (R_ig) edges of individual cells from each of the three rows. We plot the absolute difference (apparent diameter change) between motions of the two edges (ΔR) as a function of the mean displacement (R_mean). Each point in the graph corresponds to a different cell. R_mean ranged from 90-200 nm and ΔR from 20-150 nm. For all cells, R_lam and R_ig moved in phase (not shown in Figure 2-12). For OHC1 and OHC2 R_lam > R_ig, whereas, for OHC3 R_ig > R_lam. Similar to previous findings, OHC1 and OHC2 moved in phase, toward the spiral lamina, and OHC3 moved 180° out of phase, toward the spiral ligament.
Figure 2-12: Absolute difference (diameter change) between the spiral lamina edge of the cell and the spiral ligament edge of the cell, as a function of the average displacement of the two edges. Also shown is the estimated regression line, which was forced to go through zero. All data points are from the same middle turn experiment (exp. 1011, CF~4 kHz). The stimulus frequency was 60 Hz.
IV. DISCUSSION

A. Excised cochlea preparation

The major advantage of the excised cochlea preparation is that video microscopy can be used to observe the resulting responses of hundreds of cells simultaneously. This is especially important in vibration analysis, which requires the direct comparison of the relative magnitude and phase of motion of several points along the plane of interest and along different planes.

On the other hand, the use of an excised cochlea preparation poses some questions as to how the results can be related back to an intact cochlea. Some of the problems associated with the excised cochlea preparation are (1) the endocochlear potential decreases as soon as the animal is decapitated; (2) when the cochlea is opened, the fluid mass acting on the cochlear partition can present a significant load and reduce the amplitude of the vibrations at low frequencies (von Bekesy, 1960, pp. 483-484; Ufendahl et al., 1991; Cooper and Rhode, 1996); (3) during the experiment the mechanical properties of the cells can change.

When the endocochlear potential decreases the forward transduction process is compromised. As a result the feedback loop in which the OHCs are involved is reduced. This can be used to our advantage if we are interested in investigating the effect of OHC electromotility in the vibration pattern of the OC. By opening the loop, we can directly stimulate the reverse transduction process and understand its contribution to cochlear micromechanics.

Naidu and Mountain (1998) have measured the stiffness of the excised gerbil OC and found it to agree with in vivo measurements by Olson and Mountain (1991, 1994) performed on the same species. Naidu and Mountain (1998) also reported that the stiffness measurements were stable over about three hours following decapitation. It was concluded that the mechanical properties of the organ must also remain constant for this time period.

Finally, another question we need to address is how the OHCs are stimulated. We believe the most likely path for the current is to go into the cell through the apical channels and out through the basolateral membrane channels. Another possibly route for the current would be through the RL and then into the hair cell via the lateral membrane and exiting
either via the opposite lateral membrane or the basal portion of the membrane. This later route appears to be less likely due to the high resistance of the tight junctions that exist between the cells (for a review see Slepecky, 1996; also see Mountain, 1989).

In our excised cochlea preparation, if the current goes into the cell through the apical channels and out through the basolateral membrane channels, then we expect that during the depolarizing phase (positive current into SV) of our stimulus the OHCs contract. This assumption is supported by several lines of indirect evidence: (1) other studies using electrical stimulation, with similar electrode configurations showed, that during the depolarizing phase of the stimulus the BM moves towards scala vestibuli; this phase of motion occurred due to the OHCs contracting and pulling on the BM (Xue et al., 1995; Nuttall et al., 1999); (2) from our multiple plane surface views we have been able to create low resolution radial cross sections of the OC; from these new sets of images we observed that during the depolarizing phase of the stimulus OHC3 increased in diameter and moved towards the spiral ligament; (3) during the depolarizing phase of the stimulus the cells are displaced towards the base; according to our discussion of the longitudinal motion of the organ (section E and Figure 2-15 of the Discussion), this type of longitudinal displacement could only occur if the cells were contracting.

**B. Effect of tissue condition on responses**

In our early experiments, the excised cochlea was immersed in oxygenated culture medium (Leibovitz L-15) which resembled perilymph. Using this medium OHC swelling was observed in all our preparations. The swelling process was subsequently accelerated when electrical current was applied and motility was completely lost within 30 minutes. Zeddies et al. (2000) reported that cell swelling in excised mammalian preparations can be inhibited for up to 3 hours if the CI in the culture medium is replaced with a less permeable, larger anion, like lactobionate or gluconate. Gluconate was also found to be effective with lizard cochlea preparations (Aranyosi, 1999, 2002). When we used gluconate as a CI substitute, we were able to inhibit cell swelling for up to nine hours post mortem.

Cell swelling can affect both the motility of the cell as well as the response characteristics. In preparations with swollen cells OHC motility was completely lost. In a few
of these preparations motion could still be evoked, for a few minutes, if sufficiently high currents were used. On the other hand, in most experiments, motility was completely blocked. It is hard to know what the exact physiological condition of those cells was. Swollen cells might have leaky membranes, and therefore larger currents are needed to stimulate the cells.

Swollen cells from the same row, when motile, moved out of phase with each other. Probably in these cases some cells that still exhibited motility pushed around cells that did not respond and those non-responsive cells passively moved around in random directions. Similar responses have been reported in organ cultures of guinea pig cochleae in response to electric stimulation (Reuter and Zenner, 1990). Although the authors did not comment on the cell swelling, they mention that these asynchronously moving cells seemed to be partially detached from surrounding cells.

For all experiments presented in this paper the cells were in good condition. By that we mean that (1) the majority of the cells within the turn of interest were not swollen during the course of the data collection, and (2) all OHC cells in the same row moved synchronously.

C. Data repeatability and variability

During our experiments continuous current stimulation caused the preparation to deteriorate with time. The degree and the time course of this deterioration varied depending on current level. As shown in Figure 2-7 when the current was kept at a minimum for motion to be observable, the results were repeatable. In fact in some of our longest experiments, data taken about 5 hours apart had magnitudes within 2.5 dB and phases within a few degrees.

Sources of variability among different experiments could be related to (1) the distance between the electrode and the imaging location, and (2) the orientation of the imaging plane with respect to the long axis of the cell ($\gamma_\text{h}, \gamma_\text{c}$). The distance between the electrode and the imaging location depended on the amount of bone surrounding the scala media of the location of interest (Figure 2-1). Too much bone did not allow us to place the electrode very close to the location of interest, and therefore larger currents were needed to
excite the organ. The orientation of the imaging plane with respect to the long axis of the cell was adjusted manually. This course adjustment did not allow us to have a precise control on the cochlear orientation. Therefore, $\gamma_r$ and $\gamma_l$ varied among experiments and could cause variability on our estimates of the magnitude of the radial and the longitudinal components of motion.

D. Estimates of length change

Assuming that the OHC is cylindrical and that the volume of the cell is conserved during contraction, the range of percent length changes that corresponds to the apparent diameter changes shown in Figure 2-12 is about 0.7-3.6 %. In this calculation the assumptions are that the length of the OHC is 40 $\mu$m (Edge et al., 1998) and the diameter of the OHC is 8 $\mu$m (our own estimates), in the middle turn of the gerbil cochlea. This range is of the same order of magnitude as the expected length changes for isolated hair cells. For example from Santos-Sacchi (1992) the range of % length changes in guinea pig OHCs, estimated from Figure 2 of their paper, is 0.75-3% depending on the membrane potential. We expected that the loading effects of surrounding cells on the OHCs would decrease the displacement magnitude of the OHCs with respect to that in isolated OHC studies. The fact that our estimates of % length change are of the same order of magnitude as those estimated from the isolated hair cell studies, suggest that OHC motility in an intact cochlea can significantly affect OC motion.

E. OHCs pivot about the RL

Most of the data presented are from regions of interest that included either the lamina or the ligament edge of the OHCs. In Figure 2-12 we presented results from experiments where extractions from both sides of the cell were possible. The fact that both sides of the cells moved in phase coupled with our observation that the displacements of the

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cell near the RL were small, leads to the conclusion that the cells pivot with a center of rotation that is close to the RL.

Even if the cells were not contracting there would be an apparent diameter change caused by the rotation of the cell. In the following paragraphs we give a quantitative analysis of the apparent diameter change due to rotation and how it would affect our measurements of diameter change due to contraction.

In Figure 2-13 we show the motion of an OHC3 assuming that the cell pivots without contraction. The cell in this figure is illustrated in the resting state (thin line) and during maximum displacement resulting from pivoting around the top left corner of the rectangle (thick line). In the simplest case where the imaging plane, \( I_p \), is perpendicular to the long axis of the cell, the change in diameter caused by rotation, will be given by

\[
\Delta d = \Delta R = R_{\varphi} - R_{\varphi'} = d \left( \frac{1 - \cos \phi_R}{\cos \phi_R} \right)
\]

(2.16)

where \( R_{\varphi} \) and \( R_{\varphi'} \) are the radial displacement of the ligament and lamina edge of the cell, respectively, \( d \) is the diameter of the cell and \( \phi_R \) is the pivoting angle. Notice that we assume \( \Delta d = \Delta R \), due to the radial symmetry of the cell. The only unknown in equation 2.16 is \( \phi_R \), which can be calculated by

\[
\phi_R = \tan^{-1} \left( \frac{R_{\varphi}}{H} \right)
\]

(2.17)

where \( H \) is the vertical distance between the pivoting point and the location of \( I_p \). Similarly for OHC1 and OHC2,

\[
\phi_R = \tan^{-1} \left( \frac{R_{\varphi}}{H} \right).
\]

(2.18)

For the data of Figure 2-12, the range of \( R_{\varphi} \) (for OHC3) and \( R_{\varphi'} \) (for OHC1, OHC2) was 120-260 nm, and the range of \( H \) was from 0-40 \( \mu \)m. Notice that in this

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Figure 2-13: Schematic drawing of an OHC3 radial cross section. The cell is shown at rest (thin rectangle) and after it has rotated about the upper left edge (thick rectangle). The angle of rotation (pivoting angle) is indicated by $\phi_R$. d: diameter of the cell. $\Delta d$: observed change in diameter of the cell due to pivoting. $R_{\text{lig}}$: radial displacement of the ligament side edge of the cell. $R_{\text{lam}}$: radial displacement of the lamina side edge of the cell.
experiment $\gamma_r \sim 85^\circ$, therefore the maximum number for H was set to be equal to the length of the OHCs. For the middle turn of the gerbil cochlea this length was estimated to be 40 $\mu$m from the data of Edge et al. (1998). Using the above values and equation 2.17 we calculated $\Phi_r$ as a function of H. Figure 2-14 shows the results of this calculation. Depending on the location of $I_p$ (which in this experiment is equal to H), $\Phi_r$ ranges from $0.2^\circ$ to $7^\circ$ for $R_{ilig} = 120$ nm, and from $0.4^\circ$ to $14^\circ$ for $R_{ilig} = 260$ nm.

We then calculated the expected diameter change of the cell due to pivoting using equation 2.16. The only unknown in this equation was the diameter of the OHC, which from our images and also from Edge et al. (1998) was estimated to be 8 $\mu$m. The results are shown in Figure 2-14. Depending on H, $\Delta d$ ranges from 0.04 nm to 57 nm, for $R_{ilig} = 120$ nm, and from 0.17 nm to 266 nm, for $R_{ilig} = 260$ nm. Using our multiple level images, we estimated that our imaging plane was 30 $\mu$m below the RL. Therefore, in this experiment, the expected $\Delta d$ due to pivoting would range from 0.06 nm to 0.3 nm. This value is very small and below the noise level of our system. In fact, for any H > 4$\mu$m the resulting $\Delta d < 17$ nm, which is a very small value. Therefore, the difference in the motion of the two edges of the OHCs shown in Figure 2-12 (26 nm to about 150 nm) should be a reliable estimate of the diameter change due to OHC contraction.

Finally, note that our calculation of the mean displacement (Figure 2-12) corresponds to the displacement of the center of the cell during pivoting. This ranged from 75 nm to 200 nm. These numbers correspond to a range of pivot angles of $0.14^\circ$ to $0.38^\circ$ (for $V = 30$ $\mu$m). These angles are in agreement with the expected pivot angles shown in Figure 2-14, for $V = 30$ $\mu$m.

F. The longitudinal motion of the OC at low frequencies is simple.

During the depolarizing part of our stimulus, all structures moved in phase, towards the base of the cochlea. The larger longitudinal motion in the middle turn as compared to the apical turn was not expected because our anatomical measurements showed that the OHCs in both turns were oriented perpendicular to the RL. It may be that this longitudinal
Figure 2-14: Calculation of $\phi_R$ and $\Delta d$ from equations 2.17 and 2.16 using data from Figure 2-12. For each calculation we used the minimum and the maximum $R_{ig}$ to estimate a range of $\phi_R$ and $\Delta d$. 
motion results from constraints imposed by the DCs. Each OHC sits on the apical end of a DC. The DC sends a process towards the RL, that extends apically and, together with the cuticular plate of the OHCs, forms part of the RL (Figure 2-5B). Although in many species the third row DC process arches to form the outer tunnel, in gerbils it passes up to the RL as do the processes of the other two rows of DCs (Henson et al., 1983; Spicer and Schulte, 1994b). Within the cytoskeleton of the DC there are microfilaments, and microtubules, than run from the basal end of the DC to the apical end, and also extend to the RL through the DC process (for a complete list of references see Spicer and Schulte, 1993; Slepecky, 1996). This specialization of the DC cytoskeleton has been referred to as the microtubule stalk (Spicer and Schulte, 1993) and is also present in the pillar cells. In both structures, the stalk adds structural rigidity.

Based on the above information, we assume that during OHC contraction the DC process does not bend. Therefore, during contraction, we expect the OHC base to be displaced longitudinally towards the base of the cochlea. This motion is illustrated in Figure 2-15A. Note that, for simplicity, we also assumed that during OHC contraction the RL remains fixed and the DC moves in the vertical dimension due to the length change of the OHC. Even if this assumption was relaxed, and the length change of the OHC displaced both the RL and the BM, our prediction of Figure 2-15A would remain the same.

The above arguments however, do not explain the differences in the relative amplitude of the radial and longitudinal component, between the apical and middle turn. This difference was seen even between experiments where $\gamma_R$ and $\gamma_L$ were similar, and so the relative motion between the two components was expected to be similar. Spicer and Schulte (1994a) showed that there are structural differences in the DC body along the length of the cochlea. These differences are such that the DC stiffness decreases from base to apex (Spicer and Schulte, 1994a; Naidu and Mountain, 1998). If a similar argument holds for the DC process, then we expect that in more apical regions the DC process would be more compliant, and could bend during OHC contractions. Therefore in more apical regions, the OHCs would not rotate towards the basal end of the cochlea as much as they do in more basal regions (Figure 2-15B).

Besides the OHCs and DCs, other structures that also showed longitudinal displacements were the HCs and, to a smaller extent, the RL and the TM. We believe that these structures are just passively following the displacements imposed by the OHCs.
Figure 2-15: Schematic drawing of an OHC/DC complex shown at rest (grey fill, fine lines) and during maximum contraction (empty fill, thick lines). A: Expected OHC/DC displacement without bending of the DC_process. B: Expected OHC/DC displacement with bending of the DC_process.
G. The radial motion of the OC at low frequencies is complex.

1. OHC and DC motion

We present the first systematic study of the radial micromechanical motion of the OHCs as a function of level. Previous investigators have presented measurements of OHCs at a single level, usually the RL level. Vujanovic et al. (1999, 2000) measured the motion towards the apical and basal end of the OHCs in a gerbil hemicochlea preparation, but only report the transverse component of motion. Clearly, for such a complex structure as the OC, we expect that the vibration pattern of the organ in any direction cannot be resolved by measurements at one location. Our data show that the vibration of the OHC in the radial dimension is complex and depends on level.

All rows of OHCs showed small radial displacements at the level of the RL compared to levels closer to the basal end of the OHCs. The increase in the radial displacement of the OHCs as we focused from their apical to their basal end appears to be gradual. The OHC1 and OHC2 moved towards the spiral lamina while OHC3 moved towards the spiral ligament. Using our data and simple anatomic models of the OHCs, we concluded that the radial motion of the OHCs was a combination of contraction and pivoting (see section D of the Discussion). This complex motion of the OHCs seems to be the direct consequence of their anatomical location in the OC. When the cells contract our data suggest that they also pivot towards more compliant regions. For OHC1, OHC2 this direction was towards the inner tunnel and for the OHC3 towards the outer tunnel (Figure 2-16).

Previous studies using low frequency electric stimulation (Reuter and Zenner, 1990; Reuter et al., 1992), in immobilized guinea pig cochlear explants, reported large displacements in the OHC region and smaller displacements in the IHC and pillar cell region. The authors proposed a model where the contractions of OHC result in a radial shear of the RL (i.e. see Figure 8 in Reuter and Zenner, 1990). As a consequence, the apical end of the OHC shows larger radial displacement than the basal end. These data at first appear to contradict our measurements; we showed that the radial motion of the OHC increases towards its basal end. We believe that the RL motion observed in the above mentioned studies is a consequence of their methodology. Specifically, in both studies the
Figure 2.16: Schematic drawing showing the radial view of the OC. The OC is shown in two states: (1) at rest in light grey and (2) at maximum contraction, in black lines. The cartoon summarizes our findings of how the OC moves at low frequencies (details in the text). All motions have been exaggerated for visualization purposes.
BM was immobilized therefore during OHC contractions only the RL was allowed to move. Nevertheless, our data is consistent with their finding of small radial displacements in the IHC and PC region.

As we moved away from the basal end of the OHCs, the contrast of the structures decreased and we were not able to visualize the entire length of the DC. Therefore, most of our measurements are made from the apical half of the DC that surrounds the OHCs. DCs motion decreased as we focused from the apical end towards the basal end of the cell. The direction of motion of the apical end of the DC was that of the corresponding OHCs. Vujanovic et al. (1999) reported that in the hemicochlea preparation, at low frequencies, the movement of the DC was similar to that of the basal end of the OHC. This is consistent with our findings. In Figure 2-16, we illustrate the apical ends of DC2 and DC3 moving apart, but the basal ends moving together. The existence of gap junctions (Slepecky, 1996) between the lateral sides of the DC leads us to believe that DC from all rows would move together towards their basal end.

2. OPC motion

Small radial motions were sometimes seen in the OPC region near the basal end of the OHC. This motion could be bending of the middle of the OPCs due to the OHC1/DC complex pushing towards the spiral lamina.

3. HC motion

The HC region close to the OHC3 follows the amplitude and direction of motion of OHC3. At levels close to the BM the amplitude of radial HC motion decreases and the direction shifts towards the spiral lamina (see arrows in Figure 2-16). This was particularly evident in our middle turn experiments where we could clearly see the edge of the HC (Figure 2-3D) at levels close to the BM. We do not have a good explanation for the antiphasic motion between the upper half of the HC with the edge of the HC. In general, the motion of the HC seems to be the direct consequence of their location with respect to the OHC3. We are not aware of any quantitative studies on the radial motion of HC as a function of level.
4. TM and hairbundle motion

The motion of the TM was generally small compared to the motion at the basal end of the OHC, and depended on radial position. The TM motion was in the noise of our measurements at radial locations close to the IHC and the IPC. Motion increased at radial locations above the OHC hairbundles, and it was towards the spiral lamina during when the OHCs were contracting. For example the TM motion shown in Figure 2-10B, C and 2-11B, C is above the region of the OHC1 and OHC3 hairbundles. From these data it appears that the TM motion was larger in the apical turn than in the middle turn. We believe that this difference could be due to the different orientations of the cochlea between the two experiments.

In some middle turn experiments we were able to measure the motion of the TM_edge. The motion of this structure increased as we focused closer to the RL and it was towards the spiral ligament during OHC contraction. This is the same direction of motion seen for the OHC3 and HC. In the apical turn the TM_edge extended more towards the spiral ligament i.e. it appeared to be wider in the radial dimension. Therefore, measurements of the TM_edge were not possible in the apex, because the view of TM_edge was obstructed by the overlying stria vascularis. Other studies (Shah et al., 1995; Edge et al., 1998) have also reported that the distance from the spiral limbus attachment of the TM to the edge of the TM was larger in the cochlear apex than in the base.

According to our data, one possible scenario of TM motion, would be a combination of rotation about the limbal zone and stretching at the marginal band (TM_edge) to follow the motion of the HC (Figure 2-16). To further substantiate this scenario we briefly look into (1) the structural composition and mechanical properties of the TM and (2) the anatomical relation of the TM with the RL. We also discuss possible changes in the anatomy and the mechanical properties of the TM due to the composition and the pH of our bathing fluid.

The structural composition of the TM is complex and changes depending on radial location and level. The part of the TM that overlies the IHC and OHC hairbundles is called the middle zone. The middle zone of the TM is partially made of radially oriented type A fibers (Figure 2-3A) and contain type II and IX collagen (for a review see Slepecky, 1996). These types of collagen account for the TM's tensile strength and high resistance to stretch.
in the radial direction. To a first order approximation, the aggregate motion of this zone could be that of a rigid body, rotating about the limbal zone. On the other hand, in the gerbils, the marginal band of the TM (part of the TM closer to the HC, TM_edge, is very pronounced in the apical and middle turns, and seems to be amorphous (Edge et al. 1998). If the marginal band of the TM is not so rich in collagen fibers as the middle zone, then it could stretch.

To further support our argument we would need to know the relative stiffness of the marginal band with respect to the middle zone. To date, most of the stiffness measurements are done closer to the middle zone (for example Zwislocki and Cefaratti, 1989; Abnet and Freeman, 2000) due to its importance in micromechanics.

The attachment of the TM to surrounding cells, and the mechanical properties of these cells will influence the motion of the TM. Experimental evidence suggests that the middle zone of the TM attaches to the OHC stereocilia (for a review see Lim, 1980). On the other hand the attachment of the marginal band to the OC is unclear, and might differ among species, cochlear location and animal age. One possibility is that the marginal band attaches to the HC located next to the third row of OHCs, via the marginal net (reviewed in Lim, 1980). Our observation of the TM_edge moving in phase with the HC, appears to support this possibility.

The shape and mechanical properties of the TM can change dramatically depending on the composition and pH of the surrounding fluid. In our experiments the TM was surrounded by a perilymph-like solution instead of endolymph. Shah et al. (1995) found that in isolated mouse TM preparations substitution of artificial endolymph (AE) by artificial perilymph (AP) caused shrinkage of the TM. The shrinkage was due to the presence of high calcium concentrations in the AP. Note that in our AP-like medium the concentration of calcium was closer to AE, therefore we would not expect shrinkage to occur. On the other hand, Shah et al. (1995) found that the presence of high sodium (without the high calcium) caused the TM to swell but the accompanied length change was small. The fact that our TM_edge was located close to the HC indicates that in our preparation the shrinkage of the TM is also small and might not have altered the attachment of the marginal band to the HC. Also, the irregular pattern of the TM_edge (Figure 2-3A) has also been observed by Lim (1980) in many species, and was found to be present in the apical turn but not in the basal turn. Therefore, we do not expect that this irregularity is due to detachment of the TM at
these locations. Finally, the pH of our medium was adjusted to 7.3 which is within the limits of pH that would not cause big changes to the shape of the TM (Freeman et al., 1997). It is worth mentioning that any small changes in the shape of the TM might produce static deflections on the hairbundles and influence the forward transduction process (Shah et al., 1995). This is not an issue in our measurements since we expect that the forward transduction is negligible.

Therefore, we believe that in our preparation the TM overlies the OC approximately the way it overlies it in vivo, and that the TM has not suffered major shape changes due to our fluid composition. The structural composition and mechanical properties of the TM and the anatomical relation of the TM with the RL, coupled with our measurements, suggest that the possibility of the TM rotating about the limbal zone and stretching at the marginal band is quite plausible (Figure 2-16). More experiments need to be done to substantiate this hypothesis.

We found one other study where the authors measure the motion of the TM in response to electrical stimulation. Gummer et al., (1996) have used an excised guinea pig cochlea preparation and recorded the motion of the TM in both the radial and transverse direction. At low frequencies, the major component of motion of the TM is in the transverse direction i.e. perpendicular to the RL, and smaller motions are seen in the radial direction. The radial component of motion is towards the spiral lamina when the BM moves towards the scala vestibuli (see Figure 2B,3B in Gummer et al., 1996). These results appear to be consistent with our findings of the radial component of TM motion.

The investigation of the motion of the TM has been of great interest due to its influence on the motion of the IHC and OHC hairbundles. We have attempted to measure the motion of the hairbundles. In most of our experiments, motion of both the IHC and OHC hairbundles was very small and in the noise of our measurements. In general, we were not able to resolve if the motion we measured was due to hairbundle rotation or translation. Therefore, we will not be discussing those results any further.

5. BM motion

The radial motion of the BM was very small and usually in the noise of our measurements. This was partly due to the low contrast of our images at the BM level.
Motion in the transverse direction cannot be ruled out. In fact, in Figure 2-16 we have included the transverse motion observed by other investigators using electric stimulation. We view those studies complementary to ours and therefore we incorporate their results to present a more complete picture of the OC motion under low frequency electric stimulation.

Mammano and Ashmore (1993) measured the transverse motion of the Claudius’s cells (CC, next to the outermost HC), and the HC (at the level of the RL) in response to electric current, in the apical turn of excised guinea pig cochleae. The motion of the CC was taken to represent the motion of the BM at that region, and was found to be about 5.4 times less than the motion of the HC. In addition, during OHC contraction the BM moved towards the scala vestibuli whereas the RL moved towards the scala tympani. No measurements were made at other BM or RL locations. In Figure 2-16 we have included this anti-phasic motion between the BM and RL. We feel that the relative magnitude between the BM and RL motion will depend on radial location and that more experiments are needed to determine this relative motion.

Xue et al. (1993, 1995) and Nuttall et al. (1995, 1999) measured the transverse motion of several radial locations across the BM. Both studies were performed in the basal end of gerbil (Xue et al., 1993, 1995) or guinea pig (Nuttall et al., 1995, 1999) cochleae. Electrical stimulation revealed phase differences between the arcuate zone (AZ) and the pectinate zone (PZ) of the BM. During OHC contraction the AZ moved towards the scala tympani while the PZ moved towards the scala vestibuli (Figure 2-16). Nuttall et al. (1999) made measurements at several radial locations and found that the motion of the PZ was largest close to the foot of the OPC and OHC1 and decreased gradually from the region of OHC2 to CCs. In our illustration (Figure 2-16) we have included this anti-phasic motion between the AZ and PZ.

Karavitaki and Mountain (2000, 2001, 2002; also see Chapter 5 of this thesis) have shown that the efferent medial olivocochlear (MOC) fibers that cross the tunnel of Corti (TC) to innervate the OHCs, exhibit large longitudinal displacements during OHC contractions. MOC displacements were shown to be due to fluid flow in the TC. Fluid flow in the TC could be the cause of the anti-phasic motion between the AZ and PZ. Namely, during OHC contraction, the BM would move towards scala vestibuli while the RL would move towards scala tympani (Mammano and Ashmore, 1993). This would decrease the cross sectional area of the OC and cause fluid to be pushed into the TC. Some of that fluid could
cause the relatively compliant AZ (Olson and Mountain, 1991, 1994; Naidu and Mountain, 1998) to be displaced towards the scala tympani.

We therefore conclude that for stimulus frequencies lower than CF, the vibration pattern shown in Figure 2-16 is plausible. Any factor that affects OHC contractions, i.e. the frequency and the level of the input, will also have an effect in the vibration of the OC. The motion of the OC at low frequencies is important in shaping the tail region of the auditory nerve fiber (ANF) tuning curves. Stankovic and Guinan (2000) have shown that the responses of ANF at tail frequencies show phase changes with level and they are not the result of a simple coupling between the motion of the BM and the bending of the IHC hairbundles. They hypothesize that this phase change is affected by cochlear mechanics beyond the level of the BM and before the level of the synapse and could be the result of multiple modes of OC vibrations. Our interpretation of the low frequency motion of the OC supports a complex vibration and the possibility of mode shifts depending on the level of excitation, due to the level dependence of OHC contractions.

V. CONCLUSIONS

(1) Our measurements present the first systematic study on the internal micromechanical motion of the OC due to electrically evoked OHC contractions at low frequencies.

(2) The longitudinal motion of the OC is simple: all structures move in phase and towards the basal end of the cochlea, although by different amounts.

(3) The radial motion of the OC is complex. During OHC contractions:
   - the motion of the TM, RL, OPC and BM is small
   - the motion of OHC/DC complex is large at the basal end of the OHCs and decreases towards the RL and BM
   - OHC1 and OHC2 pivot about the RL toward the spiral lamina
   - OHC3 pivots about the RL toward the spiral ligament
   - HC follow the motion of OHC3
(4) The OHC percent length change is in the range of 0.7-3.6 % which is in the same order of magnitude as in the isolated OHC studies. This finding suggests that OHC motility in an intact cochlea can significantly affect OC motion.

(5) During OHC contractions fluid flows in the tunnel of Corti. This flow could be the cause of the previously observed anti-phasic transverse motion between the AZ and the PZ of the BM.

(6) The complex micromechanical motion of the OC may explain the phase changes observed in ANF responses to tail frequencies.
VI. REFERENCES


Evans, E.F. and Harrison, R.V. (1976). “Correlation between cochlear outer hair cell damage and deterioration of cochlear nerve tuning properties in the guinea-pig,” J. Physiol. 256(1), 43P-44P.


Chapter 3

Electrically-evoked organ of Corti modes of vibration in the excised gerbil cochlea

ABBREVIATIONS
ANF, auditory nerve fiber; AZ, arcuate zone; BM, basilar membrane; CC, Claudius's cell; CF, characteristic frequency; DC1, first row of Deiter's cell; DC2, second row of Deiter's cell; DC3, third row of Deiter's cell; HC, Hensen's cell; IHC, inner hair cell; MOC, medial olivocochlear; OC, organ of Corti; OHC, outer hair cell; OHC1, first row of outer hair cell; OHC2, second row of outer hair cell; OHC3, third row of outer hair cell; OPC, outer pillar cell; PZ, pectinate zone; RL, reticular lamina; ST, scala tympani SV, scala vestibuli; TC, tunnel of Corti; TM, tectorial membrane

I. INTRODUCTION

Our goal is to understand the internal micromechanical motion of the organ of Corti (OC) due to outer hair cell (OHC) contractions. We have previously demonstrated (Chapter 2 of this thesis) that at low frequencies the motion of the OC in the radial direction is complex. In this paper we extend our measurements to higher frequencies and look for
systematic differences in the frequency responses between individual cells in the OC and between the apical and middle turn of the cochlea. The ensemble of motion from individual cells is used to predict the radial vibration pattern of the OC.

The classical view of cochlear partition vibration is that the structure simply bends as shown in Figure 3-1A. This means that if you cut a radial section along the cochlear partition and look at its profile you would always see it moving in phase from end to end and either going up or down. Such models try to account for von Békésy’s experimental observations of the basilar membrane (BM) motion on excised cochlea preparations (von Békésy, 1960). Von Békésy, in 1953, postulated that a different type of vibration could occur for higher frequencies. In this case the leg of the outer pillar cells (OPC) could load the BM locally and facilitate its radial subdivision for high frequencies (Figure 3-1B). He was unable to show experimentally that such a radial vibration profile could exist. Therefore, he reached the “...theoretically important conclusion that the mechanical load concentrated on one point of the BM by the pillar of Corti is relatively small compared with the load of the surrounding fluid...”.

Accumulating evidence from multiple levels in the auditory pathway challenges the classical view of cochlear partition vibration. Auditory nerve fiber (ANF) rate-level functions, in response to pure tone stimuli, show striking nonlinearities. These nonlinearities were first reported by Kiang et al. (1969) and appear as a decrease in the firing rate and a simultaneous phase shift of about 180° at around 90 dB SPL. The part of the response before the phase shift was referred to as “Component I”, and after the phase shift as “Component II”. In an attempt to identify the cochlear structures underlying each of these two components, Liberman and Kiang (1984) collected data from acoustically traumatized animals with permanent threshold-shifts. This pathology reduced or totally eliminated component I as opposed to a more robust component II. Severe reduction of component I was correlated with threshold elevation of both the tip and the tail of the tuning curve and loss of inner hair cell (IHC) hairbundles. Therefore, component I was mostly associated with OHC activity coupled to IHC possibly through the tectorial membrane (TM). At higher levels such interactions between OHC and IHC is not present and manifests as component II.

Lin and Guinan (2000a, 2000b) reported that responses of cat ANF to high level clicks (>80 dB pSPL) show phase reversals. These phase reversals depend on the
Figure 3-1: Anatomical cartoon showing possible radial vibration patterns of the cochlear partition. (A) The partition moves in a simple bending mode. (B) The pectinate zone of the BM moves out of phase with the arcuate zone.
characteristic frequency of the ANF, the click level and the latency of the response. In
general, for characteristic frequencies (CFs) < 1 kHz, reversals were seen only for high level
clicks (> 95 dB pSPL) and for latencies about 0-5 CF periods. For CFs > 1 kHz two
separate regions of reversals were observed: (1) the short-latency (0-3 CF periods), high-level
(>95 dB pSPL) region, and (2) the long-latency (2-6 CF periods), low-level (85-100 dB
pSPL) region. These phase reversals are hypothesized to be due to the interactions of
multiple excitation drives, each drive corresponding to a single vibration mode of the
cochlear partition. For the range of CFs examined in their paper, Lin and Guinan conclude
that at least three resonant modes contribute in the responses from an intact cochlea.

Another complexity of ANF pure tone responses is the phenomenon of peak
splitting where the ANF responds to more than one phase of the stimulus (Kiang and
Moxon, 1972). In order to understand the origin of peak splitting, Mountain and Cody
(1989) measured the IHC receptor potential and found that it also exhibits peak splitting. At
that time they hypothesized that the peak splitting was of mechanical origin (also see
Mountain, 1989). Further evidence of this hypothesis came with the use of modeling
(Mountain and Cody, 1989, 1999). In their later model, Mountain and Cody (1999) assume
that the input to the IHC is the sum of three different excitation pathways each of which
could be associated with a different vibration mode of the OC. The modeling results capture
the complexity of the IHC receptor potential for most of the stimulus conditions and
provide theoretical evidence of multiple vibration modes in the OC.

Direct measurements of BM and TM vibrations support the existence of multiple
vibration modes under certain experimental conditions. Xue et al. (1993, 1995) using
electrical stimulation showed that, in live gerbils, the arcuate zone (AZ) of the BM moved
out of phase with the pectinate zone (PZ) of the BM. This phase difference between the two
zones was frequency dependent. Similar results were also reported by Nuttall et al. (1995,
1999) using electrical stimulation. This kind of anti-phasic vibration pattern among different
regions of the BM is reminiscent of that shown in Figure 3-1B and is consistent with the
idea of multiple vibration modes. On the other hand, results from in vivo experiments using
acoustic stimulation sometimes show BM phase differences (i.e. Nilsen and Russell, 1999)
and sometimes do not (i.e. Cooper, 2000). Finally, Gummer et al. (1996) using electrical
stimulation and Hemmert et al. (2000) using acoustic stimulation measured the radial and
transverse motion of the TM and using their results have argued that the TM resonates at frequencies below the CF of the measurements location.

Despite all these evidence of multiple OC vibration modes, our knowledge of the internal micromechanical motion of the OC due to OHC contractions is incomplete. Ultimately, it is this motion that shapes the input to the IHC hairbundles which in turn couples to the ANF. We have developed an excised cochlea preparation (modification of Ulfendahl et al., 1989a, b) which allowed us to image the responses of hundreds of OHCs simultaneously at acoustic frequencies and at multiple focal levels. We present data collected from 15 apical and 17 middle turn gerbil cochleae with CF ranging from 0.4-4 kHz. The CF of our measurement locations was estimated using the place-frequency map of the gerbil cochlea (Muller, 1996). We show that in response to electrically-evoked OHC contraction, the motion of the OC is complex and changes with frequency, and is qualitatively similar in both the apical and the middle turn of the gerbil cochlea.

II. METHODS

The surgical preparation of gerbil cochlea, the video microscopy system, electrical stimulation and data analysis have been described in detail in Chapter 2. Here, we briefly mention the key aspects and highlight additions or differences.

A. Surgical preparation of gerbil cochleae

Young female Mongolian gerbils were used in these experiments. The animals were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (60mg/Kg) or using a mixture of ketamine (0.16 mg/g) and xylazine (0.008mg/g). The procedures followed an institutionally approved protocol with guidelines provided by the Laboratory Animal Care Facility at Boston University.

Following decapitation both temporal bones were excised and immersed in oxygenated culture medium (Leibovitz L-15) supplemented with 5 mM D-Glc. The pH of the solution was adjusted to 7.3 at the beginning of each experiment using NaHCO₃. In later experiments, the medium was a Cl⁻ modified perilymph-like solution composed of 140
mm D-GlcA, 6.6 mM NaCl, 100 μM CaCl₂, 3 mM KCl, 5 mM NaH₂PO₄, 100 μM MgCl₂, 5 
mM D-Glc, 5 mM Hepes (298 mOsm, pH 7.3 adjusted using 1M NaOH). Both solutions 
were at room temperature (~18°C) during the experiment.

Next the tympanic membrane was removed and the cochlea was exposed. For 
experiments in the apical turn, most of the basal and middle turn and the bone covering the 
apical turn were removed. For experiments in the middle turn, the basal and apical turn of 
the cochlea was removed, and the bone covering the scala vestibuli of the middle turn was 
peeled away. Our procedure ensured that Reissner’s membrane was preserved and the 
anatomy of scala media remained intact.

B. Video microscopy system

Following dissection, the preparation was mounted on the stage of an upright 
microscope (Olympus, BX50WI). A 4x (Olympus 4x, 0.13NA) lens was used for orienting 
the cochlea and later positioning the electrodes (described in the next section). A 20x 
(Olympus 20x, 0.5 NA) or a 60x (Olympus 60x, 0.9 NA) water immersion lens with an 
additional 2x magnification was used for detailed observation of the OC in the regions of 
interest. Figure 3-2 shows the surface view of the OC at the OHC level using the 20x 
objective. The resolution of the images using this objective was 432 nm/pixel.

A CCD (Hamamatsu, C2400-77) camera was mounted on the phototube of the 
microscope. Analog contrast enhancement and brightness enhancement was accomplished 
by using an image processor (Hamamatsu, Argus-20). The output of the image processor 
was connected to an externally triggered frame grabber (Scion Corporation, AG-5) for real 
time frame capture and averaging.

C. Electrical stimulation: hardware and software

AC current was delivered through glass pipettes filled with 3M NaCl. The input 
current electrode was placed in the scala vestibuli (SV) of the turn of interest and the return
Figure 3-2: This is a high magnification surface view of the organ of Corti captured with the described video stroboscopy system at one particular phase of the stimulus (exp. 131). Several structures of interest are shown: the basal end of IHCs, the head of the pillar cells (PC), the three rows of OHCs and the area of the HC. In all our images, the radial dimension points from spiral lamina to spiral ligament and the longitudinal dimension from base to apex. Radial motions towards the spiral ligament and longitudinal motions towards the base are positive by convention. We also point to one structure of interest in this case the edge of an OHC1, used to estimate the motion of OHC1.
current electrode was placed near the former location of SV in the next more basal turn. The pipettes were sealed with agar at their tip to prevent NaCl leakage and were connected to an optically isolated constant current source. The injected current was monitored by measuring the voltage across a 100 Ω resistor in the current return path. During electrical stimulation, current levels were limited to 4 mA or less to prevent tissue damage. The voltage drop across the fluid between the electrodes was about 150mV mA. A computer controlled, Tucker-Davis Technologies (TDT), System II analog interface was used to generate the input to the current source and to store the stimulating current.

The stimuli were sine waves with frequencies from 20 Hz to 9 kHz. Movements synchronized to the stimulus frequency were captured using stroboscopic illumination. A custom made current source was used to deliver current pulses of 200 mA peak to a light emitting diode (LED, model AND190AYP) emitting more than 50 Cd at a 4° viewing angle. The LED was mounted on a holder designed to replace the light source of the microscope. The input pulses to the strobe system were generated using the TDT system. The pulses occurred at fixed phases within the period of the stimulus with duration equal to 10% of the stimulus period.

Data were collected for five or eight equally spaced, randomized phases and for two conditions: (1) with the stimulating current present and (2) with the stimulating current turned off, referred to as the “no-stimulus condition”. The no-stimulus condition gave us an estimate of the magnitude of the minimum resolvable motion of our system and also verified that the motions observed were due to the current being present. For each stimulus period, pulses occurred only at one particular phase. Thus, to collect data from eight phases the same frequency was played eight times. For each frequency/phase combination the stimulus was on for 1 minute to provide enough images for subsequent averaging. Typically, we used a 16-frame average. The video frames of interest were subsequently digitized and animations of the observed motion were created by playing the images from each phase in succession.

D. Image processing and motion estimation

Electrically-evoked motion was estimated using two-dimensional (2D) cross-correlation. Cross correlation between the two images was performed by first extracting a
portion of the image containing a feature of interest like the edge of a hair cell (Figure 3-2). The same portion was extracted from an image taken at a different stimulus phase. The extracted images were high pass filtered to improve the contrast, and interpolated to improve the spatial resolution. Cross-correlation functions were then computed with FFTs. The location of the cross correlation peak, with respect to the origin, gave us an estimate of the magnitude and direction of motion between the original images. This procedure was then repeated for all the stimulus phases to derive the time waveform of the motion. Fourier analysis was then performed on the time series to estimate the peak magnitude and the phase of motion for each frequency.

From this 2D analysis we were able to estimate motion in two directions. The first was the radial direction (referring to the axis running from spiral lamina to spiral ligament) and the second was the longitudinal direction (referring to the axis running along the organ of Corti from base to apex). By convention positive displacements are towards the spiral ligament in the radial direction, and towards the base in the longitudinal direction (Figure 3-2).

E. Estimation of signal-to-noise ratio

Several factors contributed to variability in our measurements. One is clearly real differences between the cells related to the physiological state of each cell. Such differences might show as differences in the amplitude and phase of motion of individual cells. Another factor is low frequency building vibration and drift in the cochlear holder. Finally, another source of variability is noise within the images.

In order to estimate the signal-to-noise ratio in our measurements we assumed that the measured response will be the vectorial sum of a sinusoidal signal with fixed amplitude and phase plus a noise with fixed amplitude but random phase (Figure 3-3A). The presence of noise will affect both the magnitude and the phase of the measured response. In our experiments the magnitude of the response exhibited more variability than the phase across cells. We believe that this is due to the cells differing in their sensitivity but all responding with the same phase. We therefore decided to consider the effect of the noise on just the phase of the measured response.
Figure 3-3: Estimation of the SNR from the standard deviation of the phase of our measured response. A: The measured response is the vectorial sum of the signal (S) and a randomly varying noise (N). Depending on the phase of the noise (θ), both the magnitude and the phase (ϕ) of the measured response will change. B: Standard deviation of ϕ (s_ϕ) as a function of SNR.
The phase variation of the measured response due to a randomly varying noise is given by

$$\phi = \tan^{-1} \left( \frac{\sin \theta}{SNR} \right)$$

(3.1)

where $\theta$ is the phase of the noise and $SNR$ is the signal-to-noise ratio. We solved 3.1 numerically for $\theta$ ranging from $-\pi$ to $+\pi$ and for the $SNR$ ranging from 1 to 10 and estimated the standard deviation of $\phi$ as a function of the $SNR$. The results of this calculation are plotted in Figure 3-3B. Note that when the $SNR$ is less than 1 the measured responses are below the noise level and the standard deviation of the phase is greater than 33 degrees.

Therefore, in order to estimate the $SNR$ in our experiments we calculated the standard deviation of the phase across multiple cells as a function of frequency. In our motion measurements the noise level was different for different experiments and for different cell structures. In general the noise level ranged from 10 nm to 100 nm. It is important to note that the conclusions of this work do not rely on measurements near the noise level.

III. RESULTS

A. Repeatability of measurements over time

In order to understand the short term repeatability (i.e. within tens of minutes) we interleaved two randomized frequency runs where the second run filled-in frequencies different from the first run. In Figure 3-4A,C we show the magnitude and phase of the averaged radial displacements of first row of OHC (OHC1). Note that within the 10 minutes of data collection the responses of adjacent frequency points decreases smoothly with increasing frequency without any consistent decrease in the response of every other
Figure 3-4: OHC1 peak-to-peak radial frequency responses chosen to show the data repeatability over time. Panel A,C: averaged response collected within 10 minutes (exp. 131). Panel B,D: averaged responses collected within 5 hours (exp. 1011s1 and 1011s9).
frequency point (as would be expected if the cells were compromised during the second frequency run).

In order to understand the long term repeatability we repeated the same measurement over time. In Figure 3-4B,D we show the averaged frequency responses of OHC1, recorded 5 hours apart, from about the same cochlea location. We noted a small decrease in the overall magnitude of the response but no significant difference was seen in the phase response.

In general, data repeatability was similar to that shown in Figure 3-4, for most of our preparations. The experiment was abandoned if the motion of the OHCs decreased significantly. Decrease in motion usually correlated with cell swelling or with cell disruption due to the continuous application of current.

B. Data variability within the same experiment

In Figures 3-5 and 3-6 we show the averaged radial and longitudinal responses of several OHC1 cells, from one apical (Figure 3-5) and one middle (Figure 3-6) turn experiment. We also plot the averaged response plus one standard deviation, and the averaged response minus one standard deviation. It is clear from these figures that, for any given frequency, the magnitude of both the radial and the longitudinal responses can vary among different cells, sometimes by more than 300 nm. On the other hand the phase showed little variation especially at low frequencies.

However, the general shape of the frequency response was similar for all cells and is captured in the averaged responses. Specifically, the OHC1 responses were low pass with a cutoff frequency below the CF of the measurement location. At low frequencies, during the depolarizing phase of the stimulus, the radial phase started at about 180° which by convention (Figure 3-2) means motion away from the spiral ligament, and the longitudinal phase started at about 0°, which by convention means motion towards the base. As the frequency increased, the phase accumulated such that at around 1000 Hz the phase accumulation was about 90°.

Similar variability was observed in most of our experiments and for most of the structures. Exceptions will be noted in the following sections.
Figure 3-5: Peak-to-peak radial and longitudinal frequency responses from the OHC1 of an apical turn experiment (exp. 306s2, CF~0.8 kHz). The black line with the markers indicates the average motion of 9 OHC1 and the lines without markers are the average plus and minus one standard deviation.
Figure 3-6: Peak-to-peak radial and longitudinal frequency responses from the OHC1 of a middle turn experiment (exp. 131s1, CF~3 kHz). The black line with the markers indicates the average motion of 8 OHC1 and the lines without markers are the average plus and minus one standard deviation.
C. Comparison of frequency responses among different experiments

In Figures 3-7 and 3-8 we show averaged OHC1 radial and longitudinal responses from four apical and four middle turn experiments, to illustrate the typical range of responses between experiments. The magnitudes of the displacement have been normalized by the amplitude of the stimulus current. Responses from both turns were low pass with a cutoff frequency near or below the estimated CF of the imaging location. Variability in the absolute magnitude of the radial or longitudinal responses is partly due to the relative position of the imaging location to the input current electrode. Additional variability may come from the relative angle between the imaging plane and the long axis of the cell (γ_r, for the radial direction, and γ_l, for the longitudinal direction). We have previously shown (Chapter 2 of this Thesis) that when this angle is different from 90° then the estimated motion is larger than the actual motion. These angles are as indicated in the figure captions for each experiment. Note that γ_l is approximately 90° in all experiments therefore the variability comes because of differences in γ_r which, in these experiments, ranges from 70° to 105°. For example, experiment 817 in the apical turn and experiment 212 in the middle turn showed the smallest radial displacements. In these two experiments γ_r was about 90° and according to our discussion in Chapter 2, this imaging angle would give us the smallest estimate of radial displacement.

Magnitude responses in the longitudinal direction were larger in the middle turn experiments (compare Figure 3-7B, 3-8B). For most apical turn experiments the longitudinal motion was below the noise level except for experiment 306. Note that this experiment was taken at the high frequency end of the apical turn (CF ~ 800 Hz) whereas the rest of the apical turn experiments were around the 400 Hz region. In addition, in this experiment γ_r was the largest and that could also affect the observed longitudinal displacement.

Although we saw differences in the relative magnitude of the responses among different experiments, the phase responses were about the same. During depolarization, radial OHC1 phase for both turns started at about 180°, which by convention means motion away from the spiral ligament, and dropped to about 90° at around 1000 Hz (Figure 3-7C, 3-8C). Similarly, during depolarization, longitudinal OHC1 phase started at about 0°, which by
Figure 3-7: Averaged peak-to-peak OHC1 frequency responses from four apical turn experiments. Panels A,C: radial displacement. Panels B,D: longitudinal displacement. Experiment 312s2: CF~0.4 kHz, $\gamma_R = 80^\circ$, $\gamma_L = 94^\circ$. Experiment 817s2: CF~0.4 kHz, $\gamma_R = 85^\circ$, $\gamma_L = 85^\circ$. Experiment 1202c: CF~0.4 kHz, $\gamma_R = 75^\circ$, $\gamma_L = 85^\circ$. Experiment 306: CF~0.8 kHz, $\gamma_R = 105^\circ$, $\gamma_L = 86^\circ$. 
Figure 3-8: Averaged peak-to-peak OHC1 frequency responses from four middle turn experiments. Panels A,C: radial displacement. Panels B,D: longitudinal displacement. Experiment 131: CF~3 kHz, $\gamma_R = 76^\circ$, $\gamma_L = 89^\circ$. Experiment 203: CF~3 kHz, $\gamma_R = 70^\circ$, $\gamma_L = 90^\circ$. Experiment 212: CF~4 kHz, $\gamma_R = 86^\circ$, $\gamma_L = 87^\circ$. Experiment 213: CF~4 kHz, $\gamma_R = 78^\circ$, $\gamma_L = 90^\circ$. 

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convention is motion towards the base, and dropped to about -90° at around 1000 Hz (Figure 3-7D, 3-8D).

D. Comparison of the radial and longitudinal magnitude responses among all experiments

The major difference in the motion measurements between the apical and the middle turn was that the longitudinal component of OHCs was larger in the middle turn experiments compared to the apical turn experiments. To further illustrate this point we plotted the radial component of motion as a function of the longitudinal component, for all of our experiments and for all three rows of OHCs. The result is shown in Figure 3-9 for two frequencies from each turn. For both frequencies the radial component of motion was larger than the longitudinal component of motion in the apex (Figure 3-9A), whereas the two components were of comparable magnitude in the middle turn (Figure 3-9B). The absolute magnitude of the displacements was larger at 100 Hz compared to 600 Hz, but the trend remained the same.

We did not find any significant magnitude differences between the three rows of OHCs. In Figure 3-10 we show the mean and standard deviation of the radial and longitudinal component of motion from each row of OHCs for all our apical and middle turn experiments. The four panels in this figure correspond to the four panels of Figure 3-9. The average motion of OHC1, and to a smaller extent the motion of the third row of OHCs (OHC3), appeared to be larger in the radial direction, especially in the apical turn experiments at low frequencies. This observation was not statistically significant.

We also looked at the variation in $\gamma_R$ and $\gamma_L$ for all of our apical and middle turn experiments to understand if there is any significant difference in these angles between the two turns. These angles were previously shown to affect the estimated radial and longitudinal component of motion. In Figure 3-11 we plot the histograms of $\gamma_R$ and $\gamma_L$ for all our apical and middle turn experiments. For most of our experiments both angles were close to 90°. In the apical turn experiments, $\gamma_R$ varied the most and ranged from 70° to 110°.
Figure 3-9: Averaged peak-to-peak radial versus longitudinal displacements from all our apical and middle turn preparations and for all three rows of OHCs. Individual points in each panel correspond to the average displacement of a specific structure at the indicated frequency.
Figure 3-10: Averaged peak-to-peak radial and longitudinal displacement across all our apical (panels A,C) and all our middle turn (panels B,D) preparations from two frequencies. Over-plotted is the standard deviation from the mean for each structure.

L1/L2/L3: longitudinal displacement of OHC1/OHC2/OHC3 (gray bars).
R1/R2/R3: radial displacement of OHC1/OHC2/OHC3 (black bars).
Figure 3-11: Histogram plots of the estimated $\gamma_R$ and $\gamma_L$ across all our experiments. Black bars indicate results from the apical turn, gray bars indicate results from the middle turn.
F. Comparison among structures

In addition to measurements of OHC1 motion, we also measured the motion of the second row of OHCs (OHC2), the OHC3 and the Hensen’s cells (HC). In general, the low pass frequency response characteristics, described in sections B, C of the results, were similar among all these structures for both the radial and the longitudinal components of motion. Differences among these structures were seen in the radial phase of the response. To illustrate these differences we present radial responses from a representative apical and middle turn experiment (Figure 3-12, 3-13 respectively).

One of the most prominent differences among the responses of these structures was the anti-phasic radial motion between OHC1 and OHC3. This is illustrated in panels C of both figures. During the depolarizing phase of the stimulus, OHC1 were displaced away from the spiral ligament, while OHC3 were displaced towards the spiral ligament. Also note that this anti-phasic response was independent of the relative magnitude differences among these structures. For example at low frequencies, in Figure 3-12A, the displacement of OHC1 was greater than OHC3, while in Figure 3-13A, the displacements were of similar magnitude. At higher frequencies, the displacement of OHC3 was greater than OHC1 in both turns. This change in the relative magnitude between OHC1 and OHC3 although present in the averaged response data from all of our experiments (Figure 3-10 A, C) it was not a statistically significant finding due to the large standard deviation among the experiments. The HC had similar magnitude and phase responses with the OHC3 and were therefore also moving out of phase with OHC1. This is illustrated in Figure 3-12B,D and Figure 3-13B, D for the apical and middle turn data, respectively.

Another difference between the responses of the three rows of OHCs was the phase shift sometimes seen for OHC2 at frequencies below or near the CF of the imaging location. In most of our experiments, at low frequencies, OHC2 moved in phase with OHC1. As the frequency approached CF, OHC2 showed either an abrupt phase shift toward OHC3 (Figure 3-12C) or a more gradual shift toward OHC3 (Figure 3-13C). Although this phase shift was seen in most of our experiments there were also cases where OHC2 would move in phase with OHC1 for the entire frequency range that our signal to noise ratio was large. This is shown in Figure 3-14 for an apical and a middle turn experiment. For the apical turn
Figure 3-12: Average peak-to-peak radial frequency responses from OHC1, OHC2, OHC3, HC from an apical turn experiment (exp. 312s1, s2) where a 180° phase shift in OHC2 was observed. CF~0.4 kHz, \( \gamma_R = 80^\circ \), \( \gamma_L = 94^\circ \).
Figure 3-13: Average peak-to-peak radial frequency responses from OHC1, OHC2, OHC3, HC from a middle turn experiment (exp. 213) where a 180° phase shift in OHC2 was observed. CF~4 kHz, γR = 78°, γL = 90°.
Figure 3-14: Average peak-to-peak radial frequency responses of OHC1, OHC2, OHC3 from an apical (exp. 811, CF~0.4 kHz, $\gamma_r = 85^\circ$, $\gamma_L = 93^\circ$) and a middle turn (exp. 131, CF~3 kHz, $\gamma_r = 76^\circ$, $\gamma_L = 81^\circ$) experiment where no OHC2 phase shift was observed.
experiment notice that OHC2 is moving in phase with OHC3 instead of OHC1. This was rarely the case, and a major difference in this experiment was that the cells were swollen.

We also made measurements at the TM level and the IHC hairbundle level. Motion at these levels was generally very small and below our noise level. In one of our middle turn experiments, shown in Figure 3-15, we were able to collect data from the top of the TM, the bottom of the TM close to the level of OHC1 hairbundles, the edge of the TM (near the HC), and the IHC hairbundles. We only present the radial component of motion to highlight the phase differences among the structures. The responses of OHC1 and OHC3 are included for comparison. In this experiment, the TM motion was below our noise level, except at the region close to the OHC hairbundle. At this region, during the depolarizing phase of our stimulus, the motion of the TM was away from the spiral ligament, i.e. in phase with OHC1. On the other hand, the edge of the TM moved in phase with OHC3 towards the spiral ligament. Finally, the radial motion of the IHC hairbundles was also towards the spiral ligament. We were able to measure IHC hairbundle motion in 4 middle and 3 apical turn experiments, and the radial phase at low frequencies was, for all of them, towards the spiral ligament.

In general, the motion of OHC hairbundles was below our noise level. In one apical turn experiments we were able to measure the motion of both OHC and IHC hairbundles. Both the magnitude and phase of motion of the three rows of OHC hairbundles was similar to that of the IHC hairbundles at all frequencies.

Within the OC we also looked at the responses of the OPCs, the medial olivocochlear (MOC) fibers that cross the tunnel of Corti (TC) to innervate the OHC, the three rows of Deiter's cells (DC1, DC2, DC3) and the edge of the HC at the level of the DCs. Results from these structures, for a middle turn experiment, are shown in Figure 3-16. As before we only show the radial component of motion to highlight the differences in the phase of motion between the different structures. Also note, that these results are from the same experiment shown in Figure 3-13. In panels A, C we show the response of the OPC near the RL (OPC_top), the response of the OPC near the basal end of the OHC (OPC_bottom) and the response of the MOC fibers (longitudinal component of motion). The radial motion of the OPC was towards the spiral lamina (i.e. in phase with OHC1) and it was larger near the basal end of the OHCs. We also observed large longitudinal displacements of
Figure 3-15: Average peak-to-peak radial frequency responses of the top of the TM (tm_top), the bottom of the TM next to the OHC1 (tm_bottom), the edge of the TM next to the HC (tm_edge), the IHC hairbundles (ihcs), the OHC1 and OHC3, from a middle turn experiment (exp. 118, s16 to s20).
Figure 3-16: Average peak-to-peak radial frequency responses of the OPC at the level of the RL (pc_top), the OPC at the level of the basal end of the OHC (pc_bottom), the first, second and third row of the DC (dc1, dc2, dc3) and the edge of the HC (hc_edge) at a level close to the apical end of the DC. Also shown is the longitudinal frequency response of the MOC fibers (moc). All data are from the middle turn experiment 213. The responses of the OHCs and HCs from this experiment were shown in figure 3-12.
the MOC fibers. In this experiment, when the OHC1 moved towards the spiral lamina, the MOC fibers were displaced towards the base of the cochlea.

The radial displacement of all three rows of DC was similar to each other and less than that of the corresponding OHC (see Figure 3-13A). All rows of DC moved in phase towards the spiral ligament. Finally, the motion of the edge of the HC was smaller than that of the HC shown in Figure 3-13B, and it was towards the spiral lamina. In general, it was hard to visualize the DC and the edge of the HC in the apex. Therefore the motion of these structures was not quantified.

**IV. DISCUSSION**

**A. Excised cochlea preparation**

We have previously addressed several issues pertaining to the use of an excised cochlea preparation in understanding cochlea mechanics (Chapter 2). As before we emphasize that the major advantage of the excised cochlea preparation is that it allows the observation of hundreds of cells simultaneously. Therefore, the relative motion of these cells in response to OHC electro-motility can be investigated directly.

The vibration pattern of any complex structure depends on the mechanical properties of its individual components, on the anatomical architecture of the structure, and on the type and level of excitation (Ewins, 1984). In order to relate our findings to intact cochlea preparations, we need to address how each of these factors in our excised cochlea preparation relates to intact cochleae.

Naidu and Mountain (1998) have measured the stiffness of the excised gerbil OC and found it to agree with in vivo measurements by Olson and Mountain (1991, 1994) performed on the same species. Naidu and Mountain (1998) also reported that the stiffness measurements were stable over about three hours following decapitation. It was concluded that the mechanical properties of the organ must also remain constant for this time period. The fact that the phase of our measurements also remained constant for a long period of time further enhances our belief that the mechanism that underlies these responses remained stable during our experiments.
Although we were not able to assess the structure of the organ at the sub-cellular level, we are confident that at the cellular level the anatomical architecture of the OC is very close to in vivo conditions. This was accomplished by keeping the Reissner’s membrane intact, thus preserving the anatomy of scala media for the entire turn of interest. In addition, using the Cl⁻ modified gluconate-based culture medium, we inhibited cell swelling, thus further preserving the anatomy of the OC.

The input to the intact cochlea is the pressure difference between the scala tympani and scala vestibuli. This differential pressure, in combination with the mechanical properties of the cochlea, sets up a traveling wave on the BM. Thus the BM vibrates and causes bending of the IHC and OHC stereocilia. The bending of the stereocilia changes the ionic conductance at the surface of the cells allowing positive ions to flow into the cells whose membrane then depolarizes. Depolarization causes the OHC to contract and the current hypothesis is that the phase and magnitude of this contraction further shapes the vibration pattern of the OC. We will refer to the OHC driven OC vibration as the “OHC-driven component” while the pressure driven OC vibration as the “pressure-driven component”. The relative contribution of these two components to the total vibration of the OC depends on the type and level of excitation. We previously argued that with our electrical stimulation paradigm we emphasize the OHC-driven component (Chapter 2). In the intact cochlea the contribution of the OHC appears to dominate at low sound levels, therefore we expect that the OHC-driven component would be a dominant contributor (albeit not the only one) to the total vibration pattern at low sound levels.

The final point we address is the current path that excites the OHCs and the contracting phase of OHC motility with respect to positive current into SV. Both issues have been discussed in Chapter 2. We believe the current flows into the apical membrane and out the basolateral membrane of the OHCs. This current path in combination with our electrode placement leads us to conclude that the OHC contract during the positive phase of our current stimulus.
B. Data repeatability over time

In general, we were able to record data for up to nine hours after the animal was decapitated. Within this time interval the magnitude of the responses was within a few dB and the phase within a few degrees. This was true for all the experiments were the OHCs were in good condition, after the surgery was completed. Cells were considered to be in good condition when they were not swollen and when, during electrical stimulation, they moved synchronously within the same row. Cell swelling was indirectly assessed by the size of their diameter. More detail on the effect of swelling on the responses was presented in Chapter 2.

C. Data variability

Within the same experiment, responses of individual cells of the same type varied in magnitude by more than 300 nm (Figure 3-5, 6). This variability could be partly due to the different state of individual cells and the cells surrounding each cell. Within our field of view, some cells appeared less healthy than others; for example, look at the 4th, 6th or 7th OHC1 from the bottom of Figure 3-2. Usually these cells showed less motion. Also other attributes of the cells state, like the resting potential or membrane tension could be different from one cell to the next, and could give rise to different magnitudes of motion. On the other hand, the phase of all cells was within a few degrees, suggesting that the mechanism that shapes the response of these cells was not affected and was similar for all cells.

Across different experiments, the averaged responses of individual structures also varied in magnitude. This variability was partly due to variability in the distance between the electrode and the imaging location. The distance depended on the amount of bone surrounding the scala media of the location of interest. Too much bone did not allow the electrode to be placed very close to the imaging location and therefore larger currents were needed to excite the organ. Therefore, even when we normalized the displacements by the current magnitude (Figures 3-7, 3-8) there was still variability in the absolute magnitude of the responses.
Other sources of variability between different experiments were related to (1) the angle between the imaging plane and the long axis of the OHCs and (2) the location of the imaging plane with respect to the RL. We have previously reported (Chapter 2 of this Thesis) that the angle of orientation can result in different estimates of the radial and longitudinal motion, and that the radial and longitudinal motion of OHCs increases from their apical to their basal ends. Despite all these sources of variability, it was interesting to note that the variation in magnitude from one experiment to the next was within the same order of magnitude as the one seen between individual cells in the same experiment.

In contrast to the magnitude, the phase of the response of the same structure was the same from one experiment to the next, and as stated before, this indicates that the mechanism that shapes the responses is very similar in all the experiments. Exceptions were seen in the responses of OHC2 and those will be discussed further in following sections.

D. The longitudinal motion of the OC is simple and does not change with frequency

At low frequencies, during OHC contractions all the structures moved towards the base of the cochlea. As the frequency increased the phase of all structures lagged about 90° and was generally noisier than at low frequencies. These results support our previous findings (Karavitaki and Mountain, 2002; Chapter 2) where the motion of the OC was investigated at low frequencies and at multiple focal levels. We hypothesize that the longitudinal component of motion provides a means of longitudinal coupling between adjacent sections of the OC.

During OHC contraction the MOC fibers were displaced either towards the apical or towards the basal end of the cochlea. We present an extensive analysis of this motion in Chapter 5. Our hypothesis is that during OHC contractions the RL will be pulled closer to the BM, which will decrease the volume of the spaces of Nucl. This will force some of the fluid in this space into the TC leading to longitudinal fluid flow. The MOC fibers will then be displaced longitudinally due to this flow.

Our results also show that the OHC longitudinal component of motion is larger in the middle turn than in the apical turn (Figure 3-9) with no significant difference seen
between the three rows of OHCs (Figure 3-10). In order to understand why the longitudinal component tended to be larger in the middle turn than the apical turn, we first look into anatomical differences in the orientation of the cells between the two turns. We have previously reported (Chapter 2) that, in the longitudinal direction, the OHC are oriented perpendicular to the RL in both turns. In addition, our estimates of $\gamma_L$ for each experiment (Figure 3-11B) show that the orientation of the imaging plane with respect to the long axis of the OHCs, in the longitudinal direction, was about 90° for both turns. Therefore, our estimates of the observed longitudinal displacements are about equal to the actual longitudinal displacements. We concluded that neither the anatomical orientation of the OHC with respect to RL nor with respect to our imaging plane could explain the difference in the longitudinal component among the two turns.

A different way to think about this difference would be to look at $\gamma_R$. Indeed, $\gamma_R$ was different between the two turns (Figure 3-11A), in the sense that for most of the middle turn experiments $\gamma_R$ is close to 90° whereas for the apical turn experiments $\gamma_R$ was more spread with values ranging from 70° to 110° and most of the experiments in the range between 75° and 90°. This means that the observed radial displacements in the apical turn would tend to be larger than the actual radial displacements. We conclude that, on average, in our middle turn experiments our estimates of the observed displacements are about equal to the actual displacements whereas in the apical turn we tend to overestimate the actual radial displacements.

The above conclusion still does not rule out the possibility that the longitudinal component is larger in the middle turn than in the apical turn. We have previously hypothesized (Chapter 2 of this Thesis) that the longitudinal motion of the OHCs results from anatomical constraints imposed by the DCs. As a result of this constraint, during OHC contraction, the basal end of the OHCs is displaced towards the base of the cochlea. The DC stiffness decreases from base to apex (Spicer and Schulte, 1994; Naidu and Mountain, 1998) therefore the constraint they impose to the motion of the OHC could also decrease. Therefore, the longitudinal component of motion should be less in the apical turn compared to the middle turn.

We have not been able to find any previous literature describing the longitudinal motion of the OC in response to electric stimulation. Hemmert et al. (2000) using acoustic
stimulation, have described the motion of the OC in the apical turn of the guinea pig cochlea. They used a temporal bone preparation and measured motion of the Claudius's cells (CC), HC (near the OHC3 region) and the TM. The authors concluded that none of the structures had a significant longitudinal component of motion, although the velocity trajectories shown in Figures 4B, 6B and 8B of their paper might indicate that there is a small longitudinal component of motion at frequencies closer to the CF (open ellipses).

It is difficult to directly compare the Hemmert et al. (2000) study with ours for several reasons: (1) our data emphasize the micromechanical response of the OC due to the OHC-driven component; no acoustically-induced traveling wave is present, (2) our experimental animal was a gerbil as opposed to the guinea pig in the Hemmert et al. study; differences in the anatomical orientation of the OHCs and the DC process with respect to the RL, between the two species, could affect the magnitude of the longitudinal component.

E. The radial motion of the OC is complex and changes with frequency

1. OHC, DC, HC and OPC motion

The motion of OHCs was complex and depended on frequency. We first concentrate to low frequency responses. During OHC contractions, the OHC1 moved towards the spiral lamina and the OHC3 towards the spiral ligament (Figure 3-17A). The OHC2 generally moved in phase with OHC1, although sometimes, especially in the apical turn, it moved in phase with OHC3. In general, the radial component of OHC1 and OHC3 was larger than OHC2. One reason that OHC2 would move less than the other two rows of OHCs, could be because it is more anatomically constrained than the other two rows. On the other hand, OHC1, OHC3 are located next to bigger fluid spaces and therefore their motion is less constrained. These results are consistent with our previous findings (Chapter 2). Although not directly shown in this study, the motion of the OHCs seems to be a combination of contraction and pivoting.

As the frequency increased, OHC2 often showed a phase shift of about 180°. We were able to observe the phase shift more often in the apical turn than in the middle turn. Perhaps the reason is that the phase shift in the middle turn occurred at higher frequencies.
Figure 3-17: Schematic drawing of a radial cross section of the OC summarizing our conclusions for low (A) and high (B) frequencies. For each frequency the OC is shown in two states: (1) at rest in light grey and (2) at maximum contraction, in black lines. All motions have been exaggerated for visualization purposes.
where our signal to noise ratio was small. This frequency-dependent change in the shape of vibration is characteristic of a system that can exhibit multiple modes of vibration. Depending on the type (i.e. at which part of the structure and at what shape) and the level of excitation, one or more of these modes can be excited and the resulting vibration of the structure will be the sum of the weighted modes (Ewins, 1984). This dependence of the vibration shape on the type and level of excitation could also explain why in some experiments we did not observe a phase shift in OHC2.

The motion of the HC close to the OHC3, followed the amplitude and direction of motion of the OHC3, at all frequencies. On the other hand the motion of the edge of the HC close to the level of the DCs, was smaller than the OHC3 and HC motion, and it was towards the spiral lamina. We were only able to observe the edge of the HC in our middle turn experiments. In the apical turn, the bone of the SV blocked the view of the edge of the HC. These results agree with our previous report of the HC motion at low frequencies (Karavitaki and Mountain, 2002; Chapter 2) and extend the findings to higher frequencies. We believe that the motion of the HC passively follows the motion of OHC3. We do not have a good explanation for the anti-phasic motion between the upper half of the HC with the edge of the HC.

In the middle turn experiment shown in Figure 3-13 we were also able to measure the motion of the DC. The results were shown in Figure 3-16. All three rows of DC moved in phase and towards the spiral lamina, at all frequencies. We previously showed that, at low frequencies, the top of the DC follows the motion of the corresponding OHCs (Chapter 2). The two observations are not contradictory since the measurements we presented in Figure 3-16 were towards the middle of the DC and not near the top. In fact we confirm our hypothesis that although at their top the DC move in phase with the corresponding OHCs, at their middle and bottom they should all move together, since they are interconnected by gap junctions (Slepecky, 1996).

Finally, in Figure 3-16 we showed that the motion of the OPC was in phase with the motion of the OHC1 and that the middle of the OPCs was moving more than its top. In combination with our data at low frequencies, and at multiple focal levels (Chapter 2), we confirm our hypothesis that the OPCs appear to bend at their middle. Bending could be due to the OHC/DC complex pushing towards the spiral lamina or because of the fluid flow from the spaces Nuel into the TC.
We briefly mentioned that the frequency dependent shape of vibration observed in our experiments is characteristic of a system that can exhibit multiple modes of vibration. Zhang et al. (1996) used a finite-element method (FEM) model of the OC with model parameters that matched experimental data for the compliance of individual structures. The authors used the FEM model to solve for the modal frequencies of the OC without the fluid loading and with no TM. At the first vibration mode, the motion of the OHC, HC and DC was very similar to the one supported by our data. Because there was no fluid present in this FEM model it is hard to directly compare the modal frequencies. From our data it seems that the first modal frequency is at around 400 Hz in the apical turn (as calculated from the imaginary component of the radial motion) and at around 700 Hz (or higher) in the middle turn.

In comparing our results with other studies we need to keep in mind the differences in the experimental conditions and the focal level at which the responses of individual structures are measured. Very few studies exist that have isolated the contribution of OHCs on the OC motion using electrical stimulation, at multiple frequencies. In addition, most of the studies report the transverse component of motion at the RL level and not the radial component. In a later section we present results on the transverse component of motion as complementary to our observations of radial motion. Here we concentrate on the radial component of motion reported from one other study using electrical simulation.

Reuter and Zrenner (1990) used immobilized (i.e. their BM was fixed on a Petri dish) cochlear explants and measured the electrically-induced motion of different structures at the RL level. Their data support large radial and transverse motion at the RL. We have not been able to observe such large radial motion at the RL level (Chapter 2). We believe the RL motion observed in the above mentioned studies, is partly a consequence of the immobilized BM.

2. TM, and hairbundle motion

The motion of the TM was very small and most of the time in the noise level of our measurements. We think this is partly due to the poor contrast of the TM. We were sometimes able to measure motion at the inner side of the TM, overlying the region of the OHCs. The direction of motion of the TM at that level was in phase with the OHC1, i.e.
towards the spiral lamina during OHC contractions. Larger motions were seen at the edge of the TM overlying the HC region. At that level the motion of the TM was in phase with the HC, i.e. towards the spiral ligament during OHC contractions. We have previously reported that under our experimental conditions we expect that the TM should be mostly intact and overlying the OC as in the in vivo case (Chapter 2). Our data support our previous hypothesis based on data at low frequencies, from multiple levels. That is, the TM appears to rotate about the limbal zone and stretch at the marginal band to follow the motion of the HCs. This hypothesis is still very preliminary and more data are needed at multiple locations on the TM to further support this hypothesis.

The investigation of the motion of the TM has been of great interest due to its direct influence on the transduction process. Results using acoustic stimulation (Ulfendahl, 1995; Gummer et al., 1996; Hemmert et al., 2000), electric stimulation (Gummer et al., 1996) and mechanical stimulation (Hu et al., 1999) show that at low frequencies the major component of motion of the TM is in the transverse direction i.e. perpendicular to the RL, and smaller motions are seen in the radial direction. The radial component of motion is towards the spiral lamina when the BM moves towards the scala vestibuli (i.e. see Figure 2B,3B in Gummer et al., 1996). Some investigators believe that at frequencies below the CF, the TM/OHC complex resonates in the radial direction (Zwislocki, 1986; Gummer et al., 1996; Hemmert et al., 2000). Such a resonance was not observed in our data.

Ultimately the important measure is the relative motion between the TM and the RL, since this motion shapes the stimulus to the hairbundles of both the OHCs and the IHCs. Ideally, we would like to have pairs of measurements on the RL and TM for multiple radial locations. Our experimental results are not sufficient to make any hypothesis on the relative motion of the RL and the TM. Other researchers have attempted to quantify this relative motion. One such experiment was done by Ulfendahl et al. (1995) at one radial location. Specifically the authors report acoustically induced radial and transverse motion of the TM and RL, above the region of the OHC2, in the apical turn of excised guinea pig cochleae. At low frequencies, the motion of both structures is mostly in the transverse direction, although the RL motion also has a small radial component. At higher frequencies, near the CF, the radial motion of the RL increases. A similar study was performed in the hemi-cochlea preparation (Hu et al., 1999) with very low frequency mechanical stimuli. The results showed large radial displacements above the IHC and OHC and smaller radial displacements on the
TM. Richter and Dallos (1999) using the same hemi-cochlea preparation (albeit a slightly different mechanical excitation) reported that the motion of the RL at low frequencies was mostly in the transverse direction and at frequencies near the CF the radial component of the RL increased. The authors also reported that the tips of the IHC hairbundle moved less than the RL therefore they concluded that at CF the IHC hairbundle bends.

Another way to investigate the motion of the hairbundles would be to image their motion at multiple levels. In our preparation, it has been very difficult to measure the motion of the OHC hairbundles, mostly because the contrast at that region was small. In addition, the large (+/- 5 μm) depth of focus of our imaging system biased the motion of the OHC hairbundles from out of focus information from the RL and the internal motion of the OC. In two of our earlier experiments, when we were using the L-15 medium, we were able to record motion of the OHC hairbundles (also see Karavitaki and Mountain, 1997). The motion all three rows of OHC hairbundles appeared to be towards the spiral ligament during OHC contraction. This observation appears to be in conflict with our prediction for the TM motion, which from our data we hypothesized to be towards the spiral lamina. We interpret the motion of the OHC hairbundles with caution, since we cannot determine if the motion refers to the top or the bottom of the hairbundle. In addition, since we only have measurements at one focal level, we cannot determine whether the hairbundle rotates or just translates.

It was easier to measure the motion of the IHC hairbundles, because they had greater contrast. In general the motion of the IHC hairbundles was small. The direction of motion was towards the spiral ligament, during the contracting phase of the OHC. As with the OHCs hairbundle, we interpret the motion of the IHC hairbundle with caution. Since we only have data at one level of the IHC hairbundles, we cannot determine whether the hairbundle rotates or just translates.

F. Predicted OC vibration pattern and relation to intact preparations

The predicted OC vibration pattern is shown in Figure 3-17. With our set-up we were not able to measure transverse motion so any motion shown in this direction is based on results from other investigators. We therefore devote the next few paragraphs to briefly
summarize a few measurements of transverse motion at the RL and BM levels, performed by other investigators.

Vujanovic et al. (1999, 2000) used a gerbil hemi-cochlea preparation and measured the electrically-induced motion of the OHCs at the RL and DC level. They found that the transverse component of motion at the top of the OHCs (RL level) was small compared to the bottom of the OHC (DC level) and of opposite phase. In addition, they found that the transverse motion of the OHCs was tuned. Mammano and Ashmore (1993) have also found that in response to electric stimulation, the RL at the HC region moves out of phase with the BM at the Claudius cell region. At that region the RL motion is about 5.4 times larger than the BM motion. In our illustration (Figure 3-17) we have included this anti-phasic motion between the BM and RL. We feel that the relative magnitude between the BM and RL motion will depend on radial location and that more experiments are needed, in multiple radial locations to determine this relative motion.

Several other studies have used acoustic stimulation and measured the vibration of the RL at several radial locations in the apical turn (Ulfendahl and Khanna, 1993, excised gerbil cochlea; Khanna and Hao, 1999, live guinea pig; Hao and Khanna, 2000, live guinea pig; Hemmert et al., 2000, excised guinea pig cochlea) and in the middle turn (Ulfendahl and Khanna, 1993, excised gerbil cochlea; Hemmert et al., 2000, excised guinea pig cochlea). Most of the studies agree on a general vibration pattern, namely that the transverse motion of the RL increases from the region close to the spiral lamina towards the HC. In addition, as the frequency increases the vibration amplitude increases up to a certain characteristic frequency and then it decreases again. The frequency of the peak depends on the measurement location along the length of the cochlea. All the structures at the level of the RL were found to move in phase. Khanna and Hao (1999) saw small phase differences (about 60°) at frequencies much higher than the characteristic frequency.

It is difficult to directly compare the RL motion from the electrical and the acoustic stimulation experiments. Nevertheless, results from both acoustic and electric stimulation seem to agree that OHC contraction lead to RL transverse motion. We believe that the relative magnitude and phase of RL motion with respect to OHC contractions will depend on the frequency and level of excitation. Later in this section we further address these issues.

Studies of BM motion using electrical stimulation (Xue and Mountain, 1993, 1995; Nuttall et al., 1995, 1999) showed that the two zones of the BM can move out of phase. It is
hard to know what causes the anti-phasic motion of PZ with respect to the AZ. Our hypothesis is that when the OHCs contract they decrease the OC area and as fluid is pushed out towards the TC local pressure increases. Because the AZ is very compliant (Olson and Mountain, 1991,1994; Naidu and Mountain, 1998) the increase fluid pressure might cause an expansion of the tunnel and thus push the AZ towards the ST. This hypothesis is partly supported by our finding of fluid flow in the TC (Karavitaki and Mountain, 2000, 2001; Chapter 5). At higher frequencies the fluid flow in the tunnel decreased so the motion of the AZ will also decrease. In an intact cochlea, when the frequency of the stimulus is close to the CF, then we expect that the fluid flow will mostly be distributed between adjacent longitudinal sections. This is because the wavelength of the BM traveling wave is small and therefore when one longitudinal location will be pushed towards the SV the adjacent longitudinal sections will be pushed towards ST. Therefore the fluid flow will be more local than in the low frequency region.

Phase changes along the BM are also sometimes seen in experiments using mechanical stimulation (Richter and Dallos, 1998) and acoustic stimulation (Nilsen and Russell, 1999). Nilsen and Russell (1999) recorded from the basal turn of guinea pig animals, in vivo. The magnitude of BM motion showed two peaks, one under the middle of the AZ and another, comparatively larger, under the OHC region of the PZ. The motion was low under the foot of the OPC and reached a minimum close to the spiral ligament. BM phase depended on radial location. Phase differences between the AZ and PZ ranged from 90°-180°. These results are similar to the ones using electric stimulation. The motion of the BM changed post mortem. The magnitude was largest at the foot of the OPC and decrease on either side. All structures moved in phase with a slight difference (about 30°) between the AZ and the PZ near the spiral ligament.

On the other hand, Cooper (2000) using acoustic stimulation in the basal turn of live guinea pigs and gerbils, did not see any phase reversals across the radial dimension. In addition, the amplitude of the responses was largest at the OPC and decreased gradually in either direction. Thus the in vivo responses in Cooper (2000) resembled the post mortem responses in Nilsen and Russell (1999). In fact, Cooper reports that the radial profile did not change much post mortem. One difference between these two studies is that Cooper recorded from a location which was closer to the hook region.
We present a conceptual framework that might help explain some of the above data. Under this framework, the vibration of the OC is the superposition of two components, the pressure-driven component, and the OHC-driven component. Depending on the type (electrical vs. acoustical), frequency (relative to CF), and level of excitation, the relative contribution and phase of each of these two components will change. For example, Xue et al. (1993,1995) report that when using electric stimulation they did not see the expected phase accumulation. They hypothesized that their cochlea operated in the open-loop condition i.e. that the forward transduction process was compromised. Therefore, the BM vibration that they observe is mostly due to the OHC-driven component. On the other hand, Nuttall and Ren (1995) and Nuttall et al. (1999), using rectangular current pulses, were able to set up an electrically-evoked “acoustic-like” BM traveling wave. Under these conditions they noticed that the anti-phasic motion between the two BM zones was only present at the onset and offset of their pulses and not during the ringing transient response (Figure 4 in Nuttall et al., 1999). The authors attributed this result “to the forced oscillation of the two zones experiencing the same initial pressure”.

Under our framework, the Nuttall data can be explained as follows. The frequency of the ringing transient response indicates the CF of the measurement location and is the response expected from tonal stimuli at CF. In that respect it is mostly due to the pressure-driven component. The onset and offset of the response is due to the initial contraction or elongation of the OHC in response to current. This response is mostly due to the OHC-driven component. We therefore conclude that in this region, when the OHC-driven component dominates it causes the biphase BM motion, whereas when the pressure-driven component dominates both regions of the BM move in phase.

Our experimental results emphasize that the OHC-driven component can change with frequency. That means that the vibration of the OC can consist of multiple modes. It is therefore not surprising that under certain experimental conditions there exist phase changes, and in others they do not. The contribution of multiple modes of vibration on the total vibration of the OC is not a new concept (Mountain, 1998). It has been recently hypothesized by many researchers to try and reconcile IHC receptor potential measurements (Mountain and Cody, 1999) and ANF responses (Stankovic and Guinan, 2000; Lin and Guinan, 2000a, 2000b). Our work further enhances this view.
V. CONCLUSIONS

(1) Electrically-evoked motions from the apical and middle turns of the gerbil cochlea show low pass filtering characteristics with cutoff frequencies near or below the estimated CF of our imaging location.

(2) The longitudinal motion of the OC is simple: all structures move in phase relative to each other for all frequencies, although by different amounts.

(3) The longitudinal component of motion is larger in the middle turn compared to the apical turn. We hypothesize that this is due to anatomical constraints imposed by the DCs.

(4) The radial motion of the OC is complex: changes in the relative amplitude and phase of motion near or below the estimated CF suggest the presence of multiple vibration modes.

(5) Our conceptual framework is that the vibration of the OC is the superposition of two components, the pressure-driven component and the OHC-driven component. Depending on the type and level of excitation the relative contribution of these components will change. Our experimental results emphasize the OHC-driven component and suggest that it can change with frequency giving rise to multiple vibration modes.
VI. REFERENCES


Chapter 4

The spatial variation of the OHC input and OHC output in the electrically-stimulated excised gerbil cochlea

I. INTRODUCTION

The active involvement of outer hair cells (OHC) in cochlear function has been linked to their voltage dependent length changes. These length changes were first seen in isolated OHCs stimulated by low frequency extra-cellular electric fields (Brownell et al., 1985; Kachar et al., 1986) and later in isolated OHCs studied under voltage clamp using high frequency intracellular voltages (Ashmore, 1987). Further studies in isolated OHCs have established that these length changes depend on trans-membrane potential changes (Dallos et al., 1991) and are independent of trans-membrane current changes (Santos-Sacchi and Dilger, 1988).

Although OHC length changes have been extensively studied in isolated OHCs, the question still remains on how they affect cochlear function in vivo. A few studies have shown that electrical stimulation causes motion of the basilar membrane (BM) in vivo (Xue et al., 1993, 1995; Nuttall et al., 1995, 1999). The hypothesis is that electrical stimulation directly
stimulates the OHCs thus causing them to change their length and move the BM. Still, these studies do not provide a direct measurement of OHC motility.

In general, OHC length changes have proven hard to observe in intact cochlear preparations because they are not optically accessible. We have developed an excised cochlea preparation (modified from Ulfendahl et al., 1989a, b) that allowed us to visualize the OHCs and quantify their motion in response to electrical stimulation. Using this preparation, we have shown that the motion of the organ of Corti (OC) is complex and changes with frequency (Karavitaki and Mountain, 1997, 2000, 2002; also see Chapter 2, 3). We also showed that the OHC percent length change is on the same order of magnitude as in the isolated OHC studies, and therefore can significantly affect OC motion (Chapter 2).

In our previous studies the stimulating electrode was located next to the imaging location. In this study we varied the position of the imaging location with respect to the electrode and quantified the spatial variation of the voltage generated by our current stimulus. The interpretation of the spatial variation of the voltage has been aided by the use of a one-dimensional model of cochlear electro-anatomy. This model was also used to understand the frequency dependence of the voltage across the basolateral membrane of the OHC that drives motility.

II. METHODS

The surgical preparation of gerbil cochlea, the video microscopy system, electrical stimulation and data analysis have been described in detail in Chapter 2. Here, we briefly mention the key aspects and highlight additions or differences.

A. Surgical preparation of gerbil cochleae

Young female Mongolian gerbils were used in these experiments. The animals were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (60mg/Kg) or using a mixture of ketamine (0.16 mg/g) and xylazine (0.008mg/g). The procedures followed an institutionally approved protocol and with guidelines provided by the Laboratory Animal Care Facility at Boston University.
Following decapitation both temporal bones were excised and immersed in oxygenated culture medium. The medium was a CI modified perilymph-like solution composed of 140 mM D-GlcA, 6.6 mM NaCl, 100 μM CaCl₂, 3 mM KCl, 5 mM NaH₂PO₄, 100 μM MgCl₂, 5 mM D-Glc, 5 mM Hepes (298 mOsm, pH 7.3 adjusted using 1M NaOH). The solution was at room temperature (~18°C) during the experiment.

Next the tympanic membrane was removed and the cochlea was exposed. For experiments in the apical turn, most of the basal and middle turn and the bone covering the apical turn were removed. For experiments in the middle turn, the basal and apical turn of the cochlea was removed, and the bone covering the scala vestibuli of the middle turn was peeled away. Our procedure ensured that Reissner's membrane was preserved and the anatomy of scala media remained intact.

B. Video microscopy system

Following dissection, the preparation was mounted on the stage of an upright microscope (Olympus, BX50WI). A 4x (Olympus 4x, 0.13NA) lens was used for orienting the cochlea and later positioning the electrodes (described in the next section). A 20x (Olympus 20x, 0.5 NA) or a 60x (Olympus 60x, 0.9 NA) water immersion lens with an additional 2x magnification was used for detailed observation of the OC in the regions of interest. The resolution of the images using this objective was 432 nm/pixel.

A CCD (Hamamatsu, C2400-77) camera was mounted on the phototube of the microscope. Analog contrast enhancement and brightness enhancement was accomplished by using an image processor (Hamamatsu, Argus-20). The output of the image processor was connected to an externally triggered frame grabber (Scion Corporation, AG-5) for real time frame capture and averaging.
C. Electrical stimulation: hardware and software

AC current was delivered through glass pipettes filled with 3M NaCl. We used two electrode configurations. In the first configuration the input current electrode was placed in the same turn that responses were measured and the return current electrode was placed near the former location of scala vestibuli (SV) in the next more basal turn. The cochlea excitation using this type of electrode configuration will be referred to as “local excitation.” In the second configuration the input current electrode was placed in the most basal turn, close to the basal cut of SV, and the return electrode was placed about 2 to 3 mm away from the input current electrode. The cochlea excitation using this type of electrode configuration will be referred to as “distal excitation”. In general, for both the local and the distal excitation, the exact location of the return current electrode was not crucial to evoke motility.

The pipettes were sealed with agar at their tip to prevent NaCl leakage and were connected to an optically isolated constant current source. The injected current was monitored by measuring the voltage across a 100 Ω resistor in the current return path. During electrical stimulation, current levels were limited to 4 mA or less to prevent tissue damage. The voltage drop across the fluid between the electrodes was about 150mV/mA. A computer controlled, Tucker-Davis Technologies (TDT), System II analog interface was used to generate the input to the current source and to store the stimulating current.

The stimuli were sine waves with frequencies from 20 Hz to 9 kHz. Movements synchronized to the stimulus frequency were captured using stroboscopic illumination. A custom made current source was used to deliver current pulses of 200 mA peak to a light emitting diode (LED, model AND190AYP) emitting more than 50 Cd at a 4° viewing angle. The LED was mounted on a holder designed to replace the light source of the microscope. The input pulses to the strobe system were generated using the TDT system. The pulses occurred at fixed phases within the period of the stimulus with duration equal to 10% of the stimulus period.

Data were collected for eight equally spaced, randomized phases and for two conditions: (1) with the stimulating current being present and (2) with the stimulating current turned off, referred to as the “no-stimulus condition”. The no-stimulus condition gave us an estimate of the magnitude of the minimum resolvable motion of our system and also verified
that the motions observed were due to the current being present. For each stimulus period, pulses occurred only at one particular phase. Thus, to collect data from eight phases the same frequency was played eight times. For each frequency/phase combination the stimulus was on for 1 minute to provide enough images for subsequent averaging. Typically, we used a 16-frame average. The video frames of interest were subsequently digitized and animations of the observed motion were created by playing the images from each phase in succession.

D. Image processing and motion estimation

Electrically-evoked motion was estimated using two-dimensional (2D) cross-correlation. Cross correlation between the two images was performed by first extracting a portion of the image containing a feature of interest like the edge of a hair cell. The same portion was extracted from an image taken at a different stimulus phase. The extracted images were high pass filtered to improve the contrast, and interpolated to improve the spatial resolution. Cross-correlation functions were then computed with FFTs. The location of the cross-correlation peak, with respect to the origin, gave us an estimate of the magnitude and direction of motion between the original images. This procedure was then repeated for all the stimulus phases to derive the time waveform of the motion. Fourier analysis was then performed on the time series to estimate the peak magnitude and the phase of motion for each frequency.

From this 2D analysis we were able to estimate motion in two directions. The first was the radial direction (referring to the axis running from spiral lamina to spiral ligament) and the second was the longitudinal direction (referring to the axis running along the organ of Corti from base to apex). We define positive displacements as movements towards the spiral ligament in the radial direction, and towards the base in the longitudinal direction.
E. Voltage measurements

Voltage measurements were performed using glass pipettes filled with 3M NaCl, which had the same inner diameter as the current electrodes (about 300 µm). The negative voltage electrode was placed next to the return current electrode. The positive voltage electrode was placed first next to the input current electrode and then was sequentially moved at locations away from the input current electrode. The voltage electrodes were connected to a DC-coupled differential amplifier. The output of the amplifier was connected to an analog to digital interface (AD2, Tucker-Davis Technologies) used to store the resulting waveforms.

The voltage measurements were performed at five frequencies: 60 Hz, 120 Hz, 450 Hz, 810 Hz and 1200 Hz. Measurements at higher frequencies (>1200 Hz) were not practical due to contamination from capacitive coupling between the electrodes. This contamination resulted in a magnitude increase in the measured voltage and also a phase lead with respect to the input current.

F. Cochlear electro-anatomical model

1. Model description

To relate our electrical stimuli to those used with isolated OHCs, a simple electroanatomical model was used to estimate intracochlear and intracellular potentials. The electro-anatomical model is a simplified one-dimensional finite difference model based on physiologically derived parameters. Figure 4-1 shows the first, second and last section of the model. The input to each section is the voltage in SV that results from our current stimulation. The lumped resistance $R_m$ represents the resistance of the Reissner's membrane per unit section. The lumped elements $R_v$ and $C_v$ represent the resistance and capacitance of the stria vascularis per unit section, respectively. The lumped elements $R_a$, $C_a$ and $R_b$, $C_b$ represent the apical and basal resistance and capacitance of all the OHCs per unit section.
Figure 4-1: One-dimensional cochlear electro-anatomical model. Three sections of the model are shown. All parameters as explained in the text.
The sections are coupled by the axial resistance of the SM per unit section, $R_{sm}$. The model is short circuited at both ends since in our preparation the two ends are open fluid spaces.

We used this model to predict the spatial distribution of the voltage in scala media. The model was also used to predict the spatial distribution of the voltage across the basolateral membrane ($R_b$) of the OHC.

2. **Parameter estimation**

All parameters were scaled according to the length of each model section. The length of each section was equal to $\Delta x = X/N$, where $X$ is the total length of the cochlea and $N$ is the total number of model sections. We assumed that the cochlea length was $X = 11.1$ mm (Muller, 1996), and we set $N = 100$. Furthermore, there was no spatial variation in the parameters of the model, except for the input voltage.

2.1 **Reissner's membrane**

We used Johnstone's *et al.* (1966) estimate of the resistivity of Reissner's membrane ($\rho_m = 36.8$ k$\Omega$·mm$^2$) and divided it by the Reissner's membrane area ($A_m = \Delta x \times 0.4$ mm) of each section to get an estimate of the resistance per unit section. The final value was $R_{sm} = 829$ k$\Omega$/section.

We have not been able to estimate a value for the capacitance of Reissner's membrane, and we have not included it in the model.

2.2 **Axial resistance of SM**

We estimated that the input impedance of scala media was 10 k$\Omega$/mm (Johnstone's *et al.*, 1966; Misrahay *et al.*, 1958; Xue, 1993) and multiplied it by the length of each section, $\Delta x$, to estimate the impedance per unit section. The final value was $R_{sm} = 1.1$ k$\Omega$/section.
2.3 Stria vascularis

We used Johnstone's et al. (1966) estimate of the resistivity of stria vascularis ($\rho_{sv} = 4.8$ k$\Omega$ mm$^2$) and divided it by the stria vascularis area ($A_{sv} = Ax * 0.2$ mm$^2$) of each section to get an estimate of the resistance per unit section. The final value was $R_{sv} = 216$ k$\Omega$/section. Nakajima and Mountain (2000) and Nakajima (2001) have estimated the time constant of the stria vascularis from their cochlear microphonic frequency response data to be about $\tau_{sv} \sim 0.22$ msec. Using this value we calculated the capacitance of the stria vascularis to be $C_{sv} = \tau_{sv} / R_{sv} = 1.05$ nF/section.

2.4 OHC

We used the same values as in Nakajima and Mountain (2000) and Nakajima (2001) for most of the OHC membrane parameters. The apical and basal OHC capacitances were estimated by combining data from Santos-Sacchi (1989) for the total capacitance of the cell and data from Dallos (1983) for the ratio of $C_a/C_b$. For OHCs from the middle turn of the gerbil cochlea $C_a = 2pF/OHC$ and $C_b = 21$ pF/OHC. In order to get the capacitance per unit section we multiplied the capacitance of an individual OHC by $(Ax/d)$, where $d$ is the diameter of an OHC plus the space between adjacent OHCs of the same row. We used $d = 10$ $\mu$m and multiplied each capacitance by three, since there are three rows of OHC per section. The final values were $C_a = 66$ pF/section and $C_b = 699$ pF/section.

In order to calculate the values for the apical resistance of the OHC we considered the OHC at rest. We estimated that, at rest, the total transducer channels that are open could range from 10% (Kros, 1996, in vitro data) to 50% (Russell and Sellick, 1983, in vivo data). Nakajima and Mountain (2000) and Nakajima (2001) have estimated that the maximum apical conductance of the OHCs $G_{max} = 11$ nS/OHC. Therefore $G_{rest}$ ranged from 1.1-5.5 nS/OHC. In order to calculate the conductance per section we multiplied the conductance of an individual OHC by $(3*Ax/d)$. The apical resistance per unit section was then calculated by taking the inverse of the conductance. The final value, $R_{a}$, ranged from 5.5-27.3 M$\Omega$/section.
Finally, $R_b$ was calculated based on the assumptions that $R_s >> R_b$ at rest and that the total OHC input resistance is 12 MΩ (Dallos, 1985). Therefore $R_b = 12 \text{ MΩ/OHC}$. The basal resistance per unit section was calculated by dividing the basal resistance per OHC by $(3*Δx/d)$. The final value was $R_b = 0.36 \text{ MΩ/section}$.

III. RESULTS

A. Voltage measurements in SV

Figure 4-2 shows the voltage in the fluid as a function of longitudinal distance from the input current electrode, for an apical turn experiment. The input current was 1.5 mA. We were not able to measure the voltage closer than 300 μm from the input current electrode due to the large size of the electrodes. Therefore in Figure 4-2 although the input current electrode was at 0 μm the first voltage measurement was 300 μm away. For any given frequency the voltage decreased as we moved further away from the input current electrode. The decrease was similar for all frequencies. In Figure 4-2 we plot the average voltage from all frequencies and the standard deviation. As the distance from the input current electrode increased the standard deviation also increased. The deviation was such that the voltage at distant locations increased with frequency.

We were able to fit the data using a decaying exponential of the form

$$V = Ce^{-y/\lambda}$$  \hspace{1cm} (4.1)

where, $V$ is the voltage, $C$ is a constant, $y$ is the longitudinal distance from the input current electrode and $\lambda$ is the space constant describing the rate at which the voltage decreases with longitudinal distance from the input current electrode. For the fit shown in Figure 4-2, $C = 2.8$ Volts and $\lambda = 150$ μm. The space constant indicates that the voltage will drop to 37% of its maximum value, 150 μm away from the input source.
Figure 4-2: Voltage in the fluid between the input and output current electrode as a function of longitudinal distance from the input current electrode, for an apical turn experiment. Each point represents the average voltage from all frequencies. The vertical lines above and below each point are the average value plus one standard deviation and the average value minus one standard deviation. The line is the resulting exponential fit between the measurement points.
B. OHC radial motion

Figure 4-3 shows the radial component of motion of the third row of OHCs as a function of longitudinal distance from the input current electrode, for three middle turn experiments. In general, the motion of the OHCs decreased as we moved away from the input current electrode, although the rate of decrease was different for different experiments. Nevertheless, the space constant describing the rate at which the motion decreases with longitudinal distance, was in the 0.5–1.5 mm range, across all experiments. This space constant is larger than the space constant of the voltage decrease measured in SV.

C. Model predictions

The model was simulated using the voltage measurements described in Figure 4-2, and the parameters values described in the methods sections. In Figure 4-4A,B we plot the input voltage $V_i$, the SM voltage $V_{sm}$, and the OHC basolateral membrane voltage $V_b$ as a function of longitudinal position, at a frequency lower than the cutoff frequency of the stria vascularis membrane. We show $V_b$ for two different values for the $R_s$, corresponding to 10% and 50% of the transducer channels being open. All output voltages are a scaled version of the input voltage. As the number of open channels increased, the $V_b$ increased.

Figure 4-4C, D shows the same voltages at a frequency higher than the cutoff frequency of the stria vascularis impedance. All voltages are smaller compared to their low frequency values. In addition, $V_b$ is independent of the number of open transducer channels.

Figure 4-5 shows the frequency response of $V_{sm}$ and $V_b$ at two longitudinal positions: (A) 111 μm and, (B) 444 μm from the input current electrode. For both locations, the frequency response of all voltages was low pass with a cutoff frequency around 0.8 kHz. When 10% of the transducer channels were open, $V_b$ showed a broad peak at around 0.8 kHz. This peak disappeared when more than 50% of the channels were open. The only difference between the two locations was in the absolute magnitude of the voltages. As we moved away from the input current electrode, the magnitude of all the voltages decreased by the same factor.
Figure 4-3: Average peak-to-peak OHC3 radial displacement as a function of longitudinal position relative to the input current electrode. Each panel corresponds to a different middle turn experiment.
Figure 4-4: Model results of the voltage in SM ($V_{sm}$) and the voltage across the basolateral membrane of the OHCs ($V_b$) as a function longitudinal position relative to the electrode. $V_b$ is shown for two cases: (1) when the open probability of the transducer channels is 10% ($V_{b_{10}}$) and (2) when the open probability is 50% ($V_{b_{50}}$). Also shown is the input voltage ($V_{in}$). All voltages are shown for a 10 Hz stimulus (panels A, B) and a 2000 Hz stimulus (panels C, D).
Figure 4-5: Model results for $V_{sm}$, $V_b_{-10\%}$ and $V_b_{-50\%}$ as a function of stimulus frequency. Results from two model sections are shown: (A) 111 μm and (B) 444 μm away from the input.
D. Comparison of responses between local and distal excitation

Results from four middle turn experiments, using the distal excitation configuration are shown in Figure 4-6. For simplicity, we only show responses from OHC1. We also overlap data from one of our local excitation experiments, to point on the differences among the two configurations. Note that the magnitude responses have been normalized by the amplitude of the stimulus current and the phases are plotted in a linear frequency scale.

The magnitude of the radial and longitudinal components of motion from the local excitation experiment was larger or similar to the distal excitation experiments. All frequency responses had low pass characteristics with a cutoff frequency below the CF of the imaging location.

The major difference between the local and distal excitation experiments was observed at the slope of the phase at low frequencies (<1 kHz). In particular, the slope of the phase for the distal excitation experiments was about twice as steep compared to that of the local excitation experiments. This steeper slope was present in both the radial and the longitudinal components of motion. At higher frequencies the signal to noise ratio of motion from the distal excitation experiments was small therefore we do not show the phase responses at these frequencies.

IV. DISCUSSION

A. The predicted voltage in SM decays faster than the motility

Our experimental results predicted that the space constant of the voltage decay in SV was about 150 μm (Figure 4-2). In addition, our model showed that $V_b$, the voltage that drives motility, has the same space constant as the voltage decay in SV. Therefore, we would have expected that the motion of OHCs spaced apart 150 μm in the longitudinal direction would differ by about 63%. Such difference would have been easy to detect among cells within our field of view, since the longitudinal length covered within our view was about 200
Figure 4-6: Average peak-to-peak OHC1 displacements from the middle turn of five gerbil cochlea. Experiment 131: local excitation (Lc), CF~3 kHz. Experiments 828, 1011, 1122, 1026: distal excitation (Ds), CF~4 kHz. For the local excitation the input electrode was in the SV next to the imaging location, for the distal excitation the input electrode was in the SV next to the basal cut.
μm. We have not observed such large differences in the motion of cells within 200 μm. In fact, as shown in Figure 4-3 the space constant of motility was between 0.5 to 1.5 mm.

We cannot explain the difference between the space constant of the voltage decay in SV and the space constant of motility. One possibility is that our voltage measurements do not really reflect what is happening next to the Reissner's membrane. On the other hand, the fact that motility is strong even at distances where the voltage in SV is small could indicate that OHC motility is excited with very small voltages. Another possibility is that at locations where the voltage is very high the OHC motility was saturated, and the decay in motility starts to appear at distances further away from the input current electrode.

B. The frequency response of $V_b$ resembles that of the OHC motility

The predicted low pass characteristics of $V_b$ (Figure 4-5) resemble those of the OHC motility (Figure 4-6). In our experiments we did not see the peak shown in Figure 4-5 for the case where the apical conductance was 10% of the maximum value. Our measurements were similar to the frequency response shown in Figure 4-5 for the case where the apical conductance was 50% of the maximum value. The peak in the frequency response was due to the difference in the time constant between the stria vascularis and the apical OHC membrane. As the $R_s$ decreased the difference between two time constants decreased and therefore the peak decreased.

The model predicts that at low frequencies, the frequency response of the OHCs is dominated by the electrical properties of the OHCs whereas at higher frequencies the response is dominated by the electrical properties of the stria vascularis. In our model, the stria vascularis set the operating point of the OHC potential, since their input impedance is much lower than that of the OHCs. Both of these observations imply that the frequency response of the OHC motility is limited by the frequency response of the stria vascularis.
C. Comparison between local and distal excitation responses

In our experiments, during local electrical stimulation we did not observe a traveling wave because the stimulus excited all cells, within the field of view, in phase. The phase accumulation in the frequency response appears to be due to the electrical properties of the OHCs and the stria vascularis. The model predicts that the magnitude of the motility would drop 20 dB/decade, above the cutoff frequency (Figure 4-5). This corresponds to a 45° phase accumulation near the cutoff frequency, which is similar to the phase accumulation we observe in our local excitation experiments (Figure 4-6).

However, when we attempted to stimulate the organ from a location distal from the observation region the low frequency slope of the phase data was about twice as steep as the low frequency slope from the local excitation (Figure 4-6). This difference could be due to the presence of a traveling wave or it could be due to the electrical properties of the scala media. We investigate both hypotheses.

If the phase accumulation that we see in the distal excitation is due to a traveling wave then assuming that the velocity of the traveling wave is constant and that the distance between our imaging location and the location of the electrode was about 4 mm, we estimate that the velocity of the traveling wave was about 4 m/sec. Note that in this calculation we used the low frequency slope of the phase response. For the 4 kHz region, which is the CF of our imaging location the velocity of the BM wave is estimated to be around 5-6 m/sec (Robles and Ruggero, 2001). Therefore, the phase accumulation of OHC motility in our distal excitation experiments is similar to that of the BM wave.

Another possible source of the steeper phase accumulation is the electrical properties of the scala media. Xue (1993) has approximated the voltage response of the SM to sinusoidal current injection by modeling the SM as a one-dimensional cable and deriving the cable equations. The phase response was given by

\[
\angle V_{sm} = -\frac{x}{\lambda} \left(1 + \omega^2 \tau^2 \right)^{\frac{1}{4}} \left[ \frac{1 - 1/\sqrt{1 + \omega^2 \tau^2}}{2} \right] - \frac{1}{2} \tan^{-1}(\omega \tau)
\]  

(4.2)
where $x$ is the longitudinal position with respect to the stimulus, $\lambda$ is the space constant of the SM, $\tau$ is the time constant of the cable and $\omega$ is the frequency of the stimulus. In our distal excitation experiments, $x \approx 4$ mm, furthermore we set $\omega \tau = 1$ for a 1 kHz stimulus and assuming that the time constant is given by the cutoff frequency (~1 kHz) of the scala media voltage (Figure 4-5). Finally, we assumed that $\lambda = 2$ mm (Johnstone et al., 1966). Using these values the phase response due to the cable properties of SM is about 74.5 degrees. Therefore the phase difference between our local and distal excitation experiments should have been about 74.5 degrees, at 1 kHz. From our experimental data (Figure 4-6) this phase difference ranges between 60-90 degrees, although, at 1 kHz we have previously shown (Chapter 3) that the standard deviation of our phase data ranges from 20-45 degrees. Therefore it appears that the low frequency phase accumulation in our distal excitation experiments is probably due to the cable properties of SM.

V. CONCLUSIONS

(1) The longitudinal space constant of the OHC magnitude of motion ranges between 0.5 and 1.5 mm. This space constant is larger than the space constant of the measured voltage decay in SV. We hypothesize that our measurements of SV voltage might not reflect what is happening near the Reissner’s membrane.

(2) Using a one-dimensional model of cochlear electro-anatomy we showed that, in our preparation, at low frequencies the frequency response of the OHCs is dominated by the electrical properties of the OHCs, whereas at higher frequencies the response is dominated by the electrical properties of the stria vascularis.

(3) The low frequency slope of the phase data is about twice as steep in the distal excitation paradigm compared to the local excitation paradigm. By modeling the SM as a one-dimensional cable (Xue, 1993) we propose that the additional phase accumulation in the distal excitation data is due to the cable properties of SM.
VI. REFERENCES


Chapter 5

Is the cochlear amplifier a fluid-pump?

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I. ABSTRACT

Outer hair cell (OHC) motility [Brownell, W.E., Bader, C.R., Bertrand, D. & de Ribaupierre, Y. (1985) Science 227, 194-196] is thought to play a key role in mammalian cochlear frequency selectivity and hearing sensitivity, but the mechanism of cochlear amplification is not well understood and remains a matter of controversy [for a review see Patuzzi, R. (1996) in The Cochlea, eds. Dallos, P., Popper, A.N. & Fay, R.R. (Springer-Verlag, New York), pp.186-257]. We have visualized and quantified the effects of electrically evoked OHC motility within the organ of Corti (OC) using an excised cochlear preparation. We found that OHC motility induces oscillatory fluid flow in the tunnel of Corti (TC) and that this flow is present at physiologically relevant frequencies. We also show, using a simple one-dimensional
hydromechanical model of the TC, that a fluid wave within the tunnel can travel without significant attenuation for distances larger than the wavelength of the cochlear traveling wave at its peak. These results in combination with a recent hypothesis that fluid flow within the tunnel is necessary for cochlear amplification [Hubbard, A.E., Shatz, L., Yang, Z & Mountain, D.C. (2000) in Symposium on recent developments in auditory mechanics, eds. Wada, H., Takasaka, T., Ikeda, K., Ohyama, K. & Koike, T. (World Scientific Publishing, Singapore), pp. 167-173] suggest that the function of the OHCs is to act as a fluid pump.

II. INTRODUCTION

External sounds are transformed into traveling waves on the basilar membrane (BM) and these waves peak at different places along the cochlea depending on the frequency of stimulation. Experimental evidence for the existence of such traveling waves was first provided by von Békésy's work on cadaver cochleae (1). More recent measurements on live animals confirm the existence of traveling waves and also reveal that in the peak region the wave is amplified and more sharply tuned than that observed by von Békésy (for a review see reference 2). Experimental evidence suggests that OHCs are essential for this enhanced response to occur. OHCs change length in response to changes in membrane potential (3,4) and appear to actively amplify BM vibration, a process that is often referred to as the "cochlear amplification" (5). The mechanism of cochlear amplification, however, is currently poorly understood and remains an area of active research.

In order to understand the role of electromotility in cochlear micromechanics we developed a technique to measure the mechanical responses within the OC due to OHC forces. We used an excised cochlea preparation because it allowed us to gain good visual access to the cochlear turn of interest. The tissue was stimulated electrically using sinusoidal current and the resulting motion was captured at specific phases within the stimulus period using stroboscopic video microscopy. Animations of these motions were created by playing the frozen images from each phase in succession. We then calculated the amplitude and phase of motion for each structure using a two-dimensional cross-correlation technique.

During electrical stimulation, in addition to the expected OHC movements, we were surprised to find a prominent displacement of the medial olivocochlear fibers (MOC) where
they cross the TC. We believe that when the OHCs contract, the cross sectional area of the OC decreases and fluid is pushed into the TC where it displaces the MOC fibers.

To quantify the influence of the OHC motility on fluid flow in the TC, we have measured the displacements of the MOC fibers and the OHCs. We present data from two of our fifteen middle turn experiments as well as data from the one apical turn experiment where we were able to find MOC fibers. The characteristic frequency (CF) of the measurement locations was estimated using the place-frequency map of the gerbil cochlea (6) and ranged between 0.4-4 kHz.

III. MATERIALS AND METHODS

A. Surgical preparation of gerbil cochleae

Young female Mongolian gerbils were decapitated after being deeply anaesthetized with an intraperitoneal injection of sodium pentobarbital (60mg/Kg) or using a mixture of ketamine (0.16 mg/g) and xylazine (0.008mg/g). The procedures followed an institutionally approved protocol and guidelines provided by the Laboratory Animal Care Facility at Boston University. Following decapitation both temporal bones were excised and muscular and brain tissue was removed. After opening the bulla the bones were immersed in oxygenated culture medium (Leibovitz L-15) supplemented with 5 mMol/l D-Glc. The pH of the solution was adjusted to 7.3 at the beginning of each experiment using NaHCO₃. In later experiments, the medium was a Cl⁻ modified perilymph-like solution composed of 140 mM D-GcA, 6.6 mM NaCl, 100 μM CaCl₂, 3 mM KCl, 5 mM Na₂PO₄, 100 μM MgCl₂, 5 mM D-Glc, 5 mM Hepes (298 mOsm, pH 7.3 adjusted using 1M NaOH). Both solutions were at room temperature (~18 °C) during the experiment. The later solution was formulated to improve the condition of the preparation and the viability of the cells. Using this solution we were able to collect data for a maximum of nine hours following decapitation.

The next step in the surgical procedure was to transfer the immersed bone under a dissecting scope (American Optical, AO570) where, the tympanic membrane, the malleus,
incus and parts of the semicircular canals were removed. Enough temporal bone was left intact to provide a stable means of holding the cochlea. The turn of interest was then exposed without damaging Reissner's membrane and the bone surrounding the scala vestibuli of the lower turn was removed to improve the optical path. The estimated time of this surgical procedure was 20 minutes following decapitation.

B. Video microscopy system

Following dissection, the preparation was mounted in a custom made chamber and then placed on the stage of an upright microscope (Olympus, BX50WI) sitting on a vibration-isolation table. A 20x (Olympus 20x, 0.5 NA) or a 60x (Olympus 60x, 0.9 NA) water immersion lens with an additional 2x magnification was used for detailed observation of the OC in the regions of interest. A CCD (Hamamatsu, C2400-77) camera was mounted on the phototube of the microscope. Analog contrast enhancement and brightness enhancement was accomplished by using an image processor (Hamamatsu, Argus-20). The output of the image processor was connected to an externally triggered frame grabber (Scion Corporation, AG-5) for real time frame capture and averaging.

C. Electrical stimulation set-up: hardware and software

The input current electrode was placed near scala vestibuli of the turn of interest and the return current electrode was placed near the former location of scala vestibule in the next more basal turn. AC current was delivered with glass pipettes filled with 3M NaCl. The pipettes were sealed with agar at their tip to prevent NaCl leakage and were connected to an optically isolated constant current source. During electrical stimulation, current levels were limited to 4 mA or less to prevent tissue damage. The injected current was monitored by measuring the voltage across a 100 Ω resistor in the current return path. A computer controlled, Tucker-Davis Technologies (TDT) analog interface was used to generate the input to the current source and to store the stimulating current.
The stimuli were sine waves with frequencies from 30 Hz to 9 kHz. The frequencies were approximately logarithmically spaced and were randomly presented in two groups. The first group covered the entire frequency range of interest and the second group also covered the entire frequency range and filled in additional frequencies. Movements synchronized to the stimulus frequency were captured using stroboscopic illumination. A custom made current source was used to deliver current pulses of 200 mA peak to a light emitting diode (LED). The LED was mounted on a holder designed to replace the light source of the microscope. The input pulses to the strobe system were generated using the TDT system. The pulses occurred at fixed phases within the period of the stimulus with duration equal to 10% of the stimulus period.

Data were collected for five or eight equally spaced phases and for two conditions: (1) with the stimulating current present and (2) with the stimulating current turned off, referred to as the “no-stimulus condition”. The no-stimulus condition gave us an estimate of the magnitude of the minimum resolvable motion of our system and also verified that the motions observed were due to the current being present. For most of the experiments stimulus phases were randomized. For each sample time window, pulses occurred only at one particular phase. Thus, to collect data from eight phases the same frequency was played eight times. For each frequency/phase combination the sample window was repeated to provide enough images for subsequent averaging. Typically we used a 16 frame average. The video frames of interest were subsequently digitized and animations of the observed motion were created for each frequency by playing the images from each phase in succession.

D. Image processing and motion estimation

Cross-correlation was used to estimate the displacement of different structures. Cross correlation between two images was performed by first extracting a region from the image containing a feature of interest like the edge of a hair cell or a portion of an MOC fiber. The same region was then extracted from an image taken at a subsequent stimulus phase or from the corresponding no-stimulus condition. Since the motion of interest was generally less than our 432 nm pixel size, the extracted images were interpolated in the frequency domain by zero-padding their 2-dimensional (2D) fast Fourier transform (FFT).
Using interpolation we were able to increase the resolution to 27 nm per pixel. Once the extracted images were interpolated, the 2D cross-correlation was computed in the frequency domain and the peak of the cross-correlation function in the spatial domain was used to estimate the magnitude and direction of motion between the original images. This procedure was repeated for all the stimulus phases to derive the time waveform of the motion.

Fourier analysis was then performed on the displacement time series to estimate the peak amplitude and the phase of motion for each frequency. Phase data were referenced to our sine phase stimuli. From this 2D analysis we were able to estimate motion in two directions. The first was the radial direction (referring to the axis running from spiral lamina to spiral ligament) and the second was the longitudinal direction (referring to the axis running along the OC, from base to apex).

The noise level in our data depended on the mechanical stability of the preparation and the contrast of the structures of interest in the images. Depending on the experiment and the structure imaged, the noise level varied between 10 and 100 nm.

IV. RESULTS

A. Organization

Because the OHCs are situated at an oblique angle with respect to the optical axis, their contraction can be resolved into vertical, longitudinal, and radial components (Fig. 5-1A). These components refer to optical coordinates as opposed to anatomical coordinates. Here, we present data for the longitudinal and radial components, since these are the only components that can be computed directly from our surface views of the OC (Fig. 5-1B,C,D,E). Radial and longitudinal displacements were measured for OHCs in all three rows (OHC1, OHC2, OHC3) and for the MOC fibers. The OHC measurements were taken close to their basal end (Fig. 5-1B).
Figure 5-1: Anatomical cross sections of the OC. Panel A is a cartoon of the OC emphasizing some of the major structures: the three rows of OHCs (OHC1, OHC2, OHC3), the IHCs, the inner pillar cells (IPC) and outer pillar cells (OPC), the arcuate zone (AZ) of the BM, the MOC fibers and the TC. The TC has a triangular cross sectional area and is filled with fluid. The anatomical structures that form the three sides of the tunnel are the IPC, the OPC and the AZ of the BM. The tunnel communicates with the spaces of Nuel (the fluid space that surrounds the basolateral membrane of the OHCs), via the space between the OPCs. Three axes of optical coordinates are shown: (1) the vertical axis, which is parallel to the optical axis of the microscope objective, (2) the radial axis, extending from the spiral lamina (LAM) to the spiral ligament (LIG) and (3) the longitudinal axis, extending from base to apex. Both the radial and the longitudinal axis are parallel to the surface of the objective. Panels B,C show optical cross sections at the levels close to the basal end of the OHCs and the MOC fibers, respectively. Depending on the preparation, the bottom of the image leads either towards the apex or the base of the cochlea. For the experimented illustrated in this figure the bottom of the image leads towards the base. The boxed regions of B,C are shown magnified in panels D,E respectively. Scale bar for B, 44 μm, and for D, 22 μm.
B. Middle turn experiments

In Fig. 5-2 we present the results from two of our fifteen middle turn preparations. Fig. 5-2A, B shows the average magnitude and phase of displacement for OHC1 and MOC fibers, from experiment 131R. Fig. 5-2C, D shows the results of the same structures from experiment 213R. For all of our experiments the magnitude responses of OHC2 and OHC3 were qualitatively similar to OHC1 (7) and therefore are not shown in the figure. In addition, the magnitude responses of all three rows of OHCs had displacements in both the radial and longitudinal directions that were qualitatively similar. For simplicity of presentation we show only the radial component. For all experiments the major component of the displacement for the MOC (about 90% of the total magnitude) fibers was in the longitudinal direction. Therefore, only this major component of displacement is presented here.

The magnitude responses shown in Figure 5-2A,C are low pass with a cutoff frequency below the estimated CF of our measurement location. The longitudinal displacements of the MOC fibers are similar in magnitude to or larger than the radial displacements of the OHCs. At low frequencies, the OHC1 move towards the spiral lamina and the MOC fibers move towards the base of the cochlea, during the positive half of the stimulus period.

Fluid flow in the TC was estimated by first calculating the MOC fiber velocity (Figure 5-3). For the experiments shown in Figure 5-3 the velocity ranges between about 130 and 1800 μm/sec. The velocity range across all of our experiments was between about 30 and 1800 μm/sec. The oscillatory fluid flow in the TC was then estimated by multiplying the velocity with the TC area. To estimate the TC area we approximated the TC as a triangular structure with base equal to the width of the arcuate zone (AZ) of the BM and height equal to the height of the IPC. The values of these structures at the location of our measurements were estimated from (8) and resulted in an area of $1.6 \times 10^3 \ \mu m^2$. This value is representative of the 4 kHz region where most of our data were collected. The resulting flow for the experiments shown in Figure 5-3 ranges between 208 and 2880 pl/sec and for all of our experiments the range is between 48 and 2880 pl/sec. In our excised cochlea preparation the current spread is about +/- 1.5 mm from the electrode location. Therefore, the MOC fiber
Figure 5-2: Frequency response of OHC1 (solid symbols) and MOC fibers (open symbols) for experiment 131R (panels A,B) and 213R (panels C,D). Each line represents the average of several cells of the corresponding structures. For OHC1 the peak-to-peak (PP) displacement shown is in the radial direction whereas for the MOC fibers the PP displacement is in the longitudinal direction. For both experiments zero phase for the OHCs corresponds to motion towards the spiral ligament and for the MOC fibers corresponds to motion towards the base. The CF of the imaging location is shown for each experiment.
Figure 5-3: Calculated MOC velocity for experiment 131R (black line, filled symbols) and 213R (gray line, open symbols). The CF of the imaging location is shown for each experiment. The velocity was calculated by multiplying the displacement at each frequency with the corresponding radian frequency $\omega = 2\pi f$. Data points close to the noise level (above 3 and 6 kHz for each experiment respectively) have been omitted for clarity.
displacements and flow estimates presented in this paper reflect the contribution of a large number of OHCs.

The magnitude and direction of fluid flow in the TC differ from one experiment to the other. The relative magnitude of the OHC and MOC fiber displacements depended on the distance between the electrode location and the imaging site as well as the imaging angle. The direction of flow was sometimes towards the apex and other times towards the base of the cochlea, during the positive phase of the stimulus. This difference in the direction of flow may be due to the fact that in our preparation, unlike the normal cochlea, the basal end, and in some cases, also the apical end of the TC is open.

To understand the magnitude and direction of fluid flow we measured in the same cochlea OHC and MOC fiber displacement at multiple longitudinal locations while keeping the location of the electrode fixed. Shown in Figure 5-4 are the results of these measurements for two experiments. Positive flow values indicate flow towards the apex and negative values flow towards the base of the cochlea. From panels A,C we see that as we move away from the electrode the magnitude of OHC3 displacements decreases. For the case of exp. 1011R the displacements are in the noise level at the location furthest from the electrode. From panels B,D we see a shift in the direction of flow going from base to apex at a location apical to the electrode. In addition, the absolute magnitude of flow increases as we move away from the zero flow location.

A feature we see in the data is a decrease in the OHC3 displacements and tunnel flow at locations close to the electrode (i.e. point s6 in Figure 5-4A,C and s5 in Figure 5-4B,D). Note that these were the last data points to be collected and probably the condition of OHCs has deteriorated. In our preparation when the cells deteriorate their contractions decrease and therefore less fluid flow will be generated per section.

C. Apical turn experiments

It has been very difficult to find MOC fibers in the apical turn of the gerbil cochlea. In general, we observed that the number of MOC fibers crossing the TC decreases toward the apex of the gerbil cochlea. Studies in the guinea pig cochlea have also shown that the MOC innervation decreases toward the apex (9). In one of our apical turn experiments
Figure 5-4: OHC3 total peak-to-peak displacement and resulting TC flow as a function of longitudinal position for experiments 1011R and 1026R (panel A, C and B, D respectively). The stimulus frequency is 60 Hz. Each point in the graph represents the average displacement/flow along a 200 µm cochlear length. The data were collected randomly in space and their time sequence is indicated by the legend on the right of each panel i.e. data from location s2 were collected first, data from location s3 were collected second, etc. The arrows indicate the location of the basal cut (b), the electrode (e) and the apical cut (a). The noise level for exp. 1011R is about 40 nm and for exp. 1026R is about 30 nm.
where we could clearly identify MOC fibers, the results were similar to the middle turn experiments. The displacement of the MOC fibers was similar in magnitude to that of the OHCs and, at low frequencies, during the positive half of the stimulus period, the MOC fibers moved towards the basal end of the cochlea.

V. DISCUSSION

A. What is the link between OHC contractions and MOC fibers displacement?

MOC fibers show no displacement in experiments where the OHCs show no response to electrical stimulation. This observation provides strong evidence that the displacements of the MOC fibers are closely associated with OHC contractions.

In order to understand the link between OHC contractions and MOC fibers displacement we have considered two hypotheses: (1) the longitudinal displacements of the MOC fibers could be due to the OHC contractions increasing and decreasing tension in the fibers; and (2) the MOC fibers move due to fluid flow in the TC induced by OHC contractions. If hypothesis #1 were true, then we would expect to see the shape of the MOC fibers change as the length of the OHCs changed. In addition, we would expect that the MOC fibers would exhibit significant displacement in the radial direction, and very little displacement in the longitudinal direction. Neither of these predictions was observed in our preparations. Shown in Figure 5-5 are images of three MOC fibers taken at three different time points during one cycle of the stimulus. In panel 5-5A, the MOC fibers are shown in their relaxed state when no current is applied. In panel 5-5B,C the same fibers are shown in response to a 30 Hz electrical stimulus at phase 45° and 270° respectively. In this magnified view of the MOC fibers, we see no shape change and the longitudinal displacements are much larger than the radial displacements. The large longitudinal displacements of the MOC fibers provide strong evidence for hypothesis #2. Hypothesis #2 is also consistent with the anatomy of the OC. When OHCs contract the reticular lamina will be pulled closer to the
Figure 5-5: Images of three MOC fibers taken at different times within one stimulus period. (A) No stimulus condition. (B) Phase 45°. (C) Phase 270°. The dotted line in each of the images is added as a reference point to emphasize the observation that during the stimulus period the MOC fibers are displaced longitudinally without significant shape changes. The orientation is as in figure 5-1.
BM, which will decrease the volume of the spaces of Nucl. This will force some of the fluid in this space into the TC leading to longitudinal fluid flow.

Further support for hypothesis #2 comes from experiments in which we imaged multiple regions of the cochlea along the longitudinal dimension (Figure 5-4A,C). In regions close to the stimulating electrode, we observed OHC contractions and MOC longitudinal displacements (points s2, s5, s6). In regions further from the stimulating electrode, we observed MOC displacements but the OHC contractions were in the noise level (points s3, s4). This observation indicates that fluid flow generated at one location can propagate at a more distal location.

B. Magnitude and direction of fluid flow

In order to understand the magnitude and the direction of fluid flow we used a simple one dimensional hydromechanical model of the TC (Figure 5-6). The TC is treated as an elastic tube with a cross sectional area equal to the cross sectional area of the TC. The parameters description is as in Figure 5-6 and their resulting values varied from base to apex and were based on anatomical and experimental measurements from gerbil cochlea. In our preparation the basal and apical end of the cochlea is open therefore the pressure difference at these points is zero. We represent these points in our model as short circuits. The input to the model is the fluid flow generated per section from the contractions of the OHCs (I(y)). Our hypothesis is that when OHCs contract they decrease the volume inside the OC pushing some of the fluid in that space to go inside the TC. The size of the OHC contractions depends on the stimulating current which decreases as we move away from the electrode (Figure 5-4A, B). Therefore, the contractions of the OHC and the resulting fluid flow that they generate will decrease as we move away from the electrode. We model the decrease of flow generated by the OHCs as a Gaussian function whose mean is at the location of the electrode and whose standard deviation is about 1.5 mm (our estimate of the current spread in our preparation). The output of the model is the TC flow which by convention is positive if directed towards the apex.

We solved the model analytically for low frequencies because at those frequencies our experimental signal-to-noise ratio is high and the impedance of the volume compliance
Figure 5-6: One-dimensional hydromechanical model of the TC. In this model, the fluid mass in the TC per unit length is represented by an inductor ($L_{tc}$), the tube resistance per unit length is represented by a resistor ($R_{tc}$) and the volume compliance of the $AZ$ per unit length is represented by a capacitor ($C_{az}$). These parameter values were calculated as follows: $L_{tc} = \rho / A_{tc}$, $R_{tc} = 8\sigma_{tc} / A_{tc}^2$, $C_{az}$ = from (10), where $\rho$ = density of water = $10^{12}$ gr/$\mu$m$^3$, $\sigma$ = coefficient of shear viscosity in water = $10^{-6}$ gr/$\mu$m sec, $A_{tc}$ = cross sectional area of the TC = $0.5 \times H_{tc} \times W_{tc}$, $H_{tc}$ = TC height estimated as the height of IPC from (9), $W_{tc}$ = TC width estimated as the width of $AZ$ from (9). The $H_{tc}$ varied from 55 $\mu$m to 76 $\mu$m and $W_{tc}$ varied from 42 $\mu$m to 52 $\mu$m from base to apex. The resulting values for $R_{tc}$ vary from $19 \times 10^{12}$ gr/$\mu$m$^5$ sec at the base to $6.5 \times 10^{12}$ gr/$\mu$m$^5$ sec at the apex. The values for $L_{tc}$ vary from $8.7 \times 10^{16}$ gr/$\mu$m$^5$ sec at the base to $5.1 \times 10^{16}$ gr/$\mu$m$^5$ sec at the apex.
is large and can be ignored. As shown in Figure 5-6, the flow generated from the OHCs per section is distributed towards the apical and basal end of the tunnel. The percent of flow going towards each end depends on the relative impedance looking into the tunnel in either direction. For more apical sections more of the OHC flow will become TC flow towards the apex than towards the base. The resulting TC flow is the sum of all these flows in either direction (shown by the arrows in Figure 5-6).

Shown in Figure 5-7 is the input flow generated from the OHCs (panel A) and the predicted TC flow (panel B) for two electrode locations. The predicted TC flow is sigmoidal and saturates at distances away from the electrode location. This is because at locations progressively further from the electrode the magnitude of flow generated from the OHCs decreases and therefore less flow is distributed on each side of the tunnel. The model predicts that there will be locations along the length of the cochlea where there will be large TC flow with little flow generated from the OHCs. In addition, for both locations we see a shift in the flow at a location close to the electrode. When the electrode is located close to the basal end (gray line) the point of zero flow is located apical to the electrode, whereas when the electrode is located close to the apical end (black line) the point of zero flow is located basal to the electrode. In addition the model predicts that the location of zero flow will be biased towards the location of the closest cut. Our experimental results shown in Figure 5-4 agree with both model predictions. In our data we do not see saturation in the TC flow presumably because we could not image fibers at such distal locations.

C. Relation to the intact cochlea

In the case of the intact cochlea, with acoustic stimulation, only a small section of the cochlea is maximally stimulated by any given frequency. In order to understand how far and how fast a fluid wave launched by a small group of OHCs can propagate in the TC, we used the above described TC model and chose to represent only a small section of the cochlea, i.e. 200 μm so that we can assume that the values of the chosen elements will be constant. The numerical values were specific for the 4 kHz region, where most of the physiological data were collected. The space constant, $\lambda$, defined as the distance over which the fluid wave in
Figure 5-7: Model input and output for two electrode positions (indicated by the arrows): 2 mm from the base (gray line) and 8 mm from the base (black line). OC Flow represents the flow generated from the OHCs per section and is the input to our model. The location where the flow shifts direction depends on both the electrode location and the impedance of the tunnel on each side of the electrode.
the TC will be attenuated by 37\%, was calculated from equation 5.1 and the velocity of propagation of the fluid wave, \( v \), in the TC was calculated from equation 5.2.

\[
\lambda = \text{Re} \left\{ \sqrt{\left( -\omega^2 L_c \frac{C_w}{C_{as}} \right) + \left( j\omega R_w \frac{C_{as}}{C_w} \right)} \right\}^{-1} \tag{5.1}
\]

\[
v = \omega \cdot \text{Im} \left\{ \sqrt{\left( -\omega^2 L_c \frac{C_w}{C_{as}} \right) + \left( j\omega R_w \frac{C_{as}}{C_w} \right)} \right\}^{-1} \tag{5.2}
\]

The resulting values for this region are: \( I_{nc} = 6.25 \times 10^{16} \text{ gr/\mu m}^5 \), \( R_c = 9.8 \times 10^{12} \text{ gr/sec} \mu m^5 \), \( C_{as} = \text{from (10)} = 18 \text{ \mu m}^3 \text{ sec}^2/\text{gr} \), \( H_c = 64 \text{ \mu m} \), \( W_c = 50 \text{ \mu m} \), \( A_c = 1.6 \times 10^3 \text{ \mu m}^2 \), \( \omega = 2\pi f \), \( f = \text{stimulus frequency} = 30\text{-}9000 \text{ Hz} \).

Using equation 5.1 we have estimated that around the 4 kHz cochlear region, a TC fluid wave can propagate for at least 1.3 mm before it gets attenuated to 37\% of its original amplitude. This distance is larger than the wavelength of the BM traveling wave at its peak, which is about 0.5 mm for the 4 kHz region (11). In addition, using equation 5.2 we have estimated the velocity of propagation of the TC fluid wave to be as high as 9 m/sec. This value is similar to the guinea pig BM traveling wave velocity calculated (12) to be 5-6 m/sec at the 5-9 kHz CF region using the data of Kohllöffel (13).

**D. Significance of fluid flow to cochlear micromechanics**

In most of the current cochlear models longitudinal coupling is neglected. These models are referred to as "classical models" (for a review see reference 14) and although they have been successful in replicating some experimental data, they still fail to replicate all the unique features of cochlear responses. The traveling-wave amplifier (TWAmp) model (15) was the first non-classical cochlear model that successfully simulated experimental measurements of BM motion. The model includes two modes of wave propagation, coupled by means of energy exchanged between two transmission lines via the contractions of OHCs. Hypotheses for possible anatomical correlates of the second, non-resonant, transmission line included the fluid-filled TC (16). Fluid flow in the TC provides a means of longitudinal coupling between adjacent sections of the BM.
The existence of fluid flow in the TC was first considered in de Boer's (17) formulation of the sandwich model, but the model did not address the functional role of this flow in cochlear amplification. Recently, Hubbard et al. (18) explicitly incorporated the TC in a multi-compartmental hydromechanical model of the cochlea. This model has the advantage over the TWAmpl in that it represents specific anatomical structures and thus is more realistic. Simulations with the Hubbard et al. (18) model show that if fluid flow in the TC is blocked, then the model is unable to replicate the experimental data. The fact (19) that the time course of maturation of the TC parallels the maturation of the physiological responses, provides additional evidence for the involvement of the tunnel in cochlear amplification. These non-classical models posed a challenge because, until now, there was no experimental evidence to confirm fluid flow in the TC.

Our experimental data demonstrate that the TC can support fluid flow along the length of the cochlea. The fact that OHC contractions lead to oscillatory fluid flow is not surprising. The novel aspect of our findings is that the fluid does not merely flow back and forth in the radial direction but can also have a significant component in the longitudinal direction through the TC. Using a simple hydromechanical model of the TC we showed that a fluid wave in the TC can travel without significant attenuation for distances larger than the wavelength of the cochlear traveling wave at its peak. Our findings combined with recent modeling results (18) suggest that OHCs act as fluid pumps and the resulting fluid flow is necessary for the function of the cochlear amplifier.

VI. ABBREVIATIONS

OHC, outer hair cell; OC, organ of Corti; MOC, medial olivocochlear; TC, tunnel of Corti; BM, basilar membrane; CF, characteristic frequency
VII. REFERENCES


