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The Interplay of Organ-of-Corti Vibrational Modes, not Tectorial-Membrane Resonance, sets Outer-Hair-Cell Stereocilia Phase to produce Cochlear Amplification

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Abstract

The mechanical motions that deflect outer-hair-cell (OHC) stereocilia and the resulting effects of OHC motility are reviewed, concentrating on high-frequency cochlear regions. It has been proposed that a tectorial-membrane (TM) resonance makes the phase of OHC stereocilia motion be appropriate to produce cochlear amplification, i.e. so that the OHC force that pushes the basilar membrane (BM) is in the same direction as BM velocity. Evidence for and against the TM-resonance hypothesis are considered, including new cochlear-motion measurements using optical coherence tomography, and it is concluded that there is no such TM resonance. The evidence points to there being an advance in the phase of reticular lamina (RL) radial motion at a frequency approximately $\frac{1}{2}$ octave below the BM characteristic frequency, and that this is the main source of the phase difference between the TM and RL radial motions that produces cochlear amplification. It appears that the change in phase of RL radial motion comes about because of a transition between different organ-of-Corti (OoC) vibrational modes that changes RL motion relative to BM and TM motion. The origins and consequences of the large phase change of RL radial motion relative to BM motion are considered; differences in the reported patterns of these changes may be due to different viewing angles. Detailed motion data and new models are needed to better specify the vibrational patterns of the OoC modes and the role of the various OoC structures in producing the modes and the mode transition.

Keywords

cochlear amplification; tectorial membrane; reticular lamina; organ of Corti

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1. Introduction

Outer Hair Cells (OHCs) provide the basis for cochlear amplification, the injection of energy cycle-by-cycle into the cochlear traveling wave. The motor is OHC somatic motility, a piezoelectric property in which changes in OHC transmembrane voltage produce changes in OHC length. OHC transmembrane voltage is controlled by the deflection of OHC stereocilia that set the open probability of mechanoelectric transduction (MET) channels in the stereocilia. OHC stereocilia extend from the reticular lamina (RL) with the longest stereocilia row imbedded in the tectorial membrane (TM). OHC-stereocilia deflections are produced by differential radial motion between the RL and the TM. To achieve cochlear amplification, the force on the basilar-membrane (BM) from OHC somatic motility must be applied at the correct phase relative to the phase of BM motion, i.e., so that the OHC force pushes the BM in the same direction as the BM is moving. Here we review relevant experiments and trace the development of ideas about how the correct phase of OHC stereocilia deflection comes about. We try to fit together existing data into a hypothetical framework of what motions influence the phase of OHC deflections, and we consider the effects of the resulting OHC motility, for high-frequency regions of the cochlea.

1.1. Early views of OHC stereocilia deflection and the stimulation of OHCs

The first anatomical model for how OHC stereocilia are deflected came from ter Kuile (1900). From his drawings, the OoC rotates about the base of the inner pillar cells, and the TM rotates about its attachment to the spiral limbus. With the ter-Kuile anatomy and the assumption that the OoC moves approximately like a rigid body, as the basilar-membrane (BM) moves up (toward scala vestibuli (SV)), the different pivot points of the OoC and the TM produce radial shearing between the TM and the RL, and this TM-RL radial shear deflects OHC stereocilia (Davis, 1965).

After OHC somatic motility was discovered and suggested to be the motor for cochlear amplification, it was realized that the deflection of OHC stereocilia predicted by the Davis (1965) model was in the wrong direction for the resulting OHC motility to push the BM at the right phase of BM motion to produce cochlear amplification. With the Davis model, upward BM displacement deflects OHC stereocilia in a clockwise, “excitatory” direction (Fig. 1A), which opens OHC MET channels and allows K^+ to flow into and depolarize OHCs. At frequencies above the OHC membrane RC time constant, OHC voltage, and therefore OHC contractions, follow the MET-channel current by up to 90° (Fig. 2B, diamond to square). The result is that the OHC contraction is 180° out of phase with BM velocity so that the maximum OHC contraction pulling the BM upward is when the BM has the greatest velocity downward (toward scala tympani (ST)) (Fig. 2B, double-arrow-up vs X). This is the opposite direction for producing cochlear amplification.

1.2 The TM-Resonance hypothesis

The “TM-resonance” hypothesis was originally proposed by Zwislocki (1980) and Allen (1980) to account for the difference between auditory-nerve tuning that was sharp, and BM tuning that was then thought to be broad. After the discovery of somatic motility, this hypothesis was repurposed as a way to have OHC somatic motility act at the right phase to

produce cochlear amplification for tones near the BM characteristic frequency (CF) (e.g., Gummer et al., 1996; Lukashkin et al., 2010; Sasmal and Grosh, 2019). With this hypothesis, TM radial motion (Fig. 1A, brown straight line with arrows) is influenced by a resonance of the mass of the TM and the combined stiffness of the TM attachment to OHC stereocilia plus the TM attachment to the spiral limbus. It was postulated that this TM resonance was at a frequency $\sim 1/2$ octave below the BM CF. With this hypothesis, a tone near CF is at a frequency above the TM resonance so that TM radial motion would be dominated by the TM mass and would lag BM motion by up to 180° (Fig. 2C, diamond (note the phase wrap around, i.e., 180° lag and 180° lead produce the same phase) vs. triangle – compare to Fig. 2B). The result of this TM 180° delay is that OHC stereocilia are deflected in the correct phase to produce cochlear amplification (OHC contraction pulls up when BM velocity is upward, e.g., Fig. 2C: OHC bottom motion and BM velocity are in phase). This hypothesis has been widely accepted, including in recent cochlear models (e.g., Sasmal and Grosh, 2019). We will trace the evidence for and against this hypothesis.

1.3 Historical and CF-region orientation

Ideas about how the cochlea works have changed considerably over time, and understanding previous results often requires understanding the conceptual framework being used when the results were obtained. In classic models, the cochlea acts a transmission line in which the energy in the traveling wave is carried longitudinally in the fluids surrounding the OoC, and OoC properties can be summarized by the local BM impedance (the ratio of the pressure on the BM to BM velocity) (de Boer, 1991). Cochlear amplification comes about by OHC motility injecting energy cycle-by-cycle into the traveling wave. The first good evidence for this energy injection came from measurements of basilar membrane motion interpreted by an indirect “inverse method” (de Boer and Nuttall, 2000). Recent direct measurements show that for frequencies just below the local CF, the OoC impedance is negative when measured in scala tympani near the BM, which means that the OoC is injecting energy cycle-by-cycle into the traveling wave just basal to the peak of the traveling wave (Dong and Olson, 2013). Perhaps the leading alternative to cycle-by-cycle energy injection is that OHCs act as an automatic gain control system, which was proposed to explain the sluggish response of cochlear amplification (e.g. Cooper and van der Heijden, 2016; Vavakou et al., 2019). However, the sluggish cochlear response is consistent with cycle-by-cycle energy injection into a resonant system, and furthermore an automatic gain control system doesn’t fit the data (Altoe et al., 2017). In each narrow cross-section of the OoC, OHC somatic motility pushing at the correct phase on the BM, injects a little energy into the traveling wave. The energy injected into the traveling wave accumulates as the traveling wave carries the energy apically to the CF region, where the energy is absorbed by local damping (de Boer and Nuttall, 2000; Fisher et al., 2012).

Throughout most of the years considered here, it was thought that the OoC vibrates as a unit with RL and BM motions that are similar in magnitude, and that OHC motility pushes on the BM through stiff Deiters cells. It was typically assumed that force transmission was mainly within a transverse section of the OoC, so that longitudinal motion of OoC structures could be ignored. We will present, and initially evaluate, published work using these assumptions. However, it is now well established that the RL motion is amplified relative to BM motion.

In addition, recent measurements indicate that OoC structures may have significant longitudinal motion, and that Deiters cells may not be stiff (e.g., Lee et al., 2016; Cooper et al., 2018). Unfortunately, the current data are not adequate to provide a new interpretational framework, so we use the old, possibly inaccurate, framework in Results and in the initial part of the Discussion. The divergent measurements and their implications are revisited in the later part of the Discussion where we consider issues such as (1) what makes RL motion be substantially larger than BM motion, (2) Deiters cell stiffness and its effects, and (3) longitudinal motion of OoC structures.

Historically most cochlear measurements have been from the basal turn, and the TM-resonance hypothesis was formulated with results from high-CF regions in mind. In high-CF regions, the frequencies involved in cochlear amplification are considerably *above* the corner-frequency of the OHC-membrane low-pass filter (~3 kHz in gerbils, Vavakou et al., 2019), with the result that the OHC receptor voltage lags the stereocilia current by 90°. In the Results and initial Discussion sections, the text will consider only high-CF regions. Low-CF regions will be briefly considered later in the Discussion. Note that what matters is CF and stimulus frequency relative to the OHC membrane corner frequency, not apex versus base, and that in mice the apex is a high-CF region.

2. Results: Measurements that shed light on OHC stimulation

2.1 TM motion in opened cochleae supported the TM-Resonance hypothesis

Measurements of TM motion in two dimensions (2D) in opened, apical turns of excised guinea-pig cochleae provided the first direct evidence for radial TM motion (Gummer et al., 1996). In responses to sound, beads on top of the TM showed both radial and transverse motions, and these motions had different amplitudes and phases. Gummer et al. (1996) interpreted the data as showing that TM radial-motion had a resonance about ½ octave below the BM CF, and that the results supported the TM-resonance hypothesis. However, Gummer et al.'s (1996) measurements were made with an unsealed hole in the cochlear apex that allowed the fast-pressure wave from stapes motion to create an OoC motion response in the cochlear region near the hole (see Cooper and Rhode, 1996). This fast-wave response would have interfered with the response from the normal slow, traveling-wave motion, and evidence for this is the big dip in the response between the supposed TM and BM resonance frequencies in Figure 3 of Gummer et al. (1996), a dip that is accounted for by cancellation of the fast and slow responses. Since the measurement is the result of the interference of two responses, it is difficult to interpret. Furthermore, Gummer et al. (1996) didn't measure BM motion and didn't show the phase of TM motion relative to BM motion or RL motion. Overall, the Gummer et al. (1996) data provide, at most, weak support for the TM-resonance hypothesis, but these data are widely quoted and often treated as if they show that the hypothesis is correct.

Measurement of the TM response phase was reexamined by the same research group in Hemmert et al., (2000) who made cochlear motion measurements in excised guinea-pig cochleae similar to Gummer et al. (1996), but with the fast-wave motion artifact reduced or removed computationally. From measurements in one guinea pig, they showed 2D TM motion in which the motion perpendicular to the RL was largest and the motion in the radial

RL direction was small at low frequencies, but grew with frequency and near CF reached a maximum about 1/2 the amplitude of the perpendicular motion with a phase difference between the motions that reached to 60°. The data of Hemmert et al. (2000) show the presence of a second mode of motion, but the phase change was much less than is expected from the TM resonance hypothesis and does not support this hypothesis. Overall, the importance of the Gummer et al. (1996) and Hemmert et al. (2000) data is that they show that sound stimulation can produce radial TM motion that behaves differently than transverse TM motion, i.e. that two modes of motion are present.

2.2 TM mechanical properties cast doubt on the TM-Resonance hypothesis

Experiments on excised mouse TMs have shown that the TM is viscoelastic at frequencies 20 Hz to 20 kHz where it acts like a heavily damped spring (Ghaffari et al., 2007; Gu et al., 2008). The resulting high damping for radial TM vibrations argues against the TM-resonance model, particularly against the TM system having a sharp radial resonance and behaving as a mass at CF throughout hearing frequencies, as originally envisioned (Zwislocki, 1980; Allen, 1980). Whether a damped TM resonance could be sharp enough to account for the sharpness of the phase change found by Dong and Olson (2013) (see section 2.4) is unclear. More data and modeling are needed regarding the mechanical properties of attached TMs.

From measurements in excised TMs, it was found that the TM can carry longitudinal traveling waves of radial motion (Ghaffari, et al. 2007). Since the TM shear stiffness is comparable to the stiffness of the OHC stereocilia, radial deflections of OHC stereocilia can be expected to produce radial TM motion, and this motion could be carried apically in a TM traveling wave. Supporting this, separate TM versus BM traveling waves have been found in *in-vivo* measurements (Lee et al. 2015). Thus, TM radial vibration at one place can be influenced both by vibrations of local OHC stereocilia and by stereocilia vibrations from more basal OHCs. These motions must combine in complex ways that depend on how much TM radial motion is produced by OHC stereocilia deflections, and how much the TM radial-motion traveling wave is carried apically, which depends on the speed and spatial rate of decay of the TM traveling wave (Ghaffari, et al., 2007).

Support for the idea that TM traveling waves substantially affect BM and OoC motion comes from experiments on genetically-modified TMs. Mice with the *Tectb* gene knocked out had reduced cochlear sensitivity along with the unusual property that their BM tuning was *sharper* than usual, i.e. BM tuning curves and suppression-tuning curves of auditory-nerve responses were about 2/3 as wide in *Tectb* knock-out cochleae as in normal cochleae (Russell et al., 2007). This finding was surprising because usually reductions of BM sensitivity like those in *Tectb* knock-out mice are accompanied by *wider* BM tuning curves. When the physical properties of TMs from *Tectb* knock-out mice were measured, it was found that the spatial rate of decay of the longitudinal propagation of TM radial waves was reduced to about 2/3 of the original value, almost the same amount by which *Tectb* BM tuning is sharper (Ghaffari et al. 2010). The data are consistent with the hypothesis that the sharpness of BM tuning is controlled, in part, by the spatial rate of decay of longitudinal propagation of TM radial vibrations (also see Lamb and Chadwick, 2014). These data

suggest that longitudinal propagation of TM radial vibrations play a role in setting the phase of OHC stereocilia deflections to produce cochlear amplification. However, other aspects of the TM changes in *Tectb* knock-out mice may also play a role such as the reduction in stiffness in the transverse direction (Lemons et al., 2019). Sharpening of cochlear mechanical tuning was also found in passive OoC vibrations from mice with degraded OHC stereocilia (Dewey et al. 2018); this sharpening may also be due to reduced longitudinal spread via TM waves and/or to reduced TM-OoC coupling.

The above experiments were done on mice but it seems likely that they apply to other mammals including humans. Measurements on the TMs from fresh human temporal bones show that human TMs have viscoelastic properties very similar to those of mice (Farrahi et al., 2016).

2.3 Measurements of TM motion conflict with the TM-Resonance hypothesis

Optical coherence tomography (OCT) has allowed cochlear motions to be measured without opening the cochlea, thereby avoiding fast-wave contamination of OoC motions. Of most interest here are measurements from live mice made from two viewing angles so that the OoC motions could be calculated into radial and transverse components (Lee et al., 2016). TM motion consisted of a transverse component that was similar to BM motion in amplitude and phase, as well as a radial component that had its greatest amplitude at a frequency above the BM CF and very little phase change relative to BM motion as stimulus frequency changed below CF. The finding of little change in the phase of TM radial motion at frequencies below CF directly contradicts the original TM resonance hypothesis which postulated a 180° phase change at a frequency below CF. Nonetheless, these experiments found that the OHC stereocilia deflection was in the correct phase to produce cochlear amplification because the top part of the OoC near the RL showed a phase *advance* relative to BM and TM motion (i.e. radial motion toward the Hensen's cells occurs in the RL before it occurs in the TM). This suggests that the correct phase for cochlear amplification is achieved not by a resonance and phase lag of the TM, but by a phase advance of RL radial motion. In interpreting these experiments, some caveats are in order, in particular, the OoC volume included in each measurement was large (e.g. 8.4 µm lateral and 11.4 µm axial resolution) so that the motion measurement may have included a mix of divergent motions from different nearby structures, e.g. the RL and the tops of the OHCs plus some part of Hensen's cells. However, the TM measurements are affected very little by this problem and the lack of a substantial TM phase change conflicts with the TM-resonance hypothesis. Dewey et al. (2018) also measured TM radial movement in the mouse 10 kHz region and found no evidence for a TM resonance below CF. It is interesting that Ulfendahl et al. (1995), using confocal laser heterodyne interferometry and two viewing angles in a guinea-pig isolated-temporal-bone preparation with a large hole in the apical cochlear bone, concluded that TM motion was only transverse and the TM-RL radial shear was produced by radial motion of the RL, which is consistent with the results of Lee et al. (2016). Also, in a hemicochlea preparation, vibrating the BM transversely produced mostly radial vibrations at the RL, but the TM showed transverse motion like the BM with very little radial motion (Hu et al., 1999). Overall, there is considerable experimental evidence against a below-CF resonance in TM radial motion and there is evidence for radial RL motion.

2.4 Measurement of a phase change associated with cochlear amplification

A phase change associated with cochlear amplification has been measured by simultaneously applying a combination of techniques in the base of gerbils with healthy cochleae (Dong and Olson, 2013). A probe in ST next to the BM measured both ST pressure and BM motion, while an attached electrode measured the local cochlear voltage response, which for low-level sounds is attributable to the current flowing through the nearby OHCs. At frequencies below about 0.7 CF, ST pressure, BM displacement, and local OHC current were all approximately in phase. As frequency was increased, at about 0.7 CF there was an abrupt phase advance in OHC current so that it led BM displacement by more than $\frac{1}{4}$ cycle, which Dong and Olson (2013) concluded is consistent with OHC motility being at a phase that can produce cycle-by-cycle injection of energy into the BM traveling wave and cause cochlear amplification. Although this conclusion used a value for the phase change from the OHC membrane time constant that might have been too high (see Vavakou et al., 2019), Dong and Olson (2013) found that, at frequencies near CF, the local BM impedance had a negative real part, which shows that there was a net energy injection into the traveling wave, i.e. there was cochlear amplification. These measurements imply that the phase difference between RL and TM motion changed abruptly at a frequency near 0.7 CF, so that OHC stereocilia deflections had a phase lead relative to BM displacement, but it doesn't tell whether the phase of RL, TM or both motions changed. The abrupt phase advance in OHC current occurred for both low and high-level tones, as well as after amplification was removed by a suppressor tone, so the phase transition appears to be due primarily to passive properties of the cochlea (Dong and Olson, 2013; 2016). Nonetheless, the suppressor did have an effect on the phase advance, which implies that the phase advance, while primarily arising from the passive mechanics, is affected by the cochlear amplifier.

3. Discussion

3.1 How is the phase change associated with cochlear amplification produced?

Here we try to fit together the data reviewed above to determine what physical mechanisms lead to the OHC stereocilia deflection phase that produces cochlear amplification. Based on Dong and Olson (2013), we conclude that for low frequencies, BM, TM, and RL phases are all approximately the same, but at frequencies 0.7 CF to CF, the phase of movement at the top of the OoC changes so that RL phase is $>1/4$ cycle ahead of TM phase, which deflects OHC stereocilia in a phase that produces cochlear amplification. Dong and Olson's (2013; 2016) data did not specify whether the phase of TM or RL radial motion changed with stimulation frequency relative to BM motion, but based on Lee et al. (2016) and other data, we assume that TM radial phase changes very little relative to BM motion at frequencies below the BM CF. Accepting the finding of little phase change in TM radial motion below CF, we look to RL motion for a phase change. Lee et al. (2016, Figure 4E) found that, at sound frequencies just below CF, TM radial phase lagged RL radial phase - but the cause of this was primarily an advance in RL radial phase relative to BM phase (Lee et al. 2016, Figure 4, panel C vs. D). *This points to the phase change that brings about cochlear amplification as a change in the phase of radial motion at the top of the OoC, i.e. at the RL, relative to TM and BM motion.* Thus, the phase transition occurs within the OoC, and is not from a TM radial resonance (brown straight lines with arrows in Fig. 1B vs. in Fig. 1A).

Although the above conclusion conflicts with the interpretation of Gummer et al. (1996) that there was a radial-motion resonance of the TM at a frequency near 0.7 CF, it does not conflict with Gummer et al.'s data. Gummer et al. reported a difference between the phases of TM radial versus TM transverse motion, but did not reference these phases to BM motion; furthermore the normal TM motions were obscured in their experiments by motion produced by the fast pressure wave. Gummer et al. (1996) also measured TM transverse motion with local electrical stimulation and observed motion changes about $\frac{1}{2}$ octave below the BM CF that they interpreted as showing a resonance of the TM, but this motion could have come from a resonance anywhere in the OoC, e.g. from the change of radial motion at the top of the OoC communicated to the TM. In a related experiment, Hemmert et al. (2000) showed TM radial motion that lagged TM transverse motion, but the change in TM radial-motion phase was only 40° from $1\frac{1}{2}$ octaves below CF to above CF, which is much less than expected from the TM-resonance hypothesis. Another argument for there being a TM resonance has been that it would explain the point of insensitivity between the tip and tail of auditory-nerve-fiber tuning curves (Allen, 1980), but measured TM motions show no evidence of a TM resonance at the tip-tail transition frequency (Lee et al., 2016), and the tip-tail transition can be explained by a transition in IHC-drive from RL-TM radial shear to fluid flow due to RL-TM gap changes (Guinan, 2012). In summary, observations stated as evidence for a TM radial resonance have other explanations and there is no good evidence for there being a TM radial resonance.

Although the OoC had been thought to move as a unit, many recent studies have reported differential motion within the OoC, in particular, that RL motion is substantially different from BM motion (Chen et al., 2011; Zha et al., 2012; Gao et al., 2014; Ramamoorthy et al., 2014; Lee et al., 2016; Warren et al., 2016; Cooper et al., 2018). Differential motion within the OoC is consistent with the interpretation that: (1) the OoC vibrates in complex modes with each mode having a unique pattern of amplitude and phase as a function of stimulation frequency, and (2) the mix of these modes changes at a frequency ~ 0.7 CF so that at frequencies between 0.7 CF and CF, the resulting RL-TM phase causes OHC stereocilia deflections to be at a phase that brings about cochlear amplification. Other evidence for a change in OoC vibrational modes is provided by the BM traveling wave, which shows an abrupt transition between long wavelengths at low frequencies and short wavelengths near CF (van der Heijden and Cooper, 2018). The abrupt wavelength transition suggests a change in the wave propagation mode consistent with a transition in the OoC vibrational mode that is dominant in long-wave vs. in short-wave regions. This evidence from the BM traveling wave suggests that the transition at ~ 0.7 CF in which OoC mode is dominant at the RL involves all aspects of cochlear motion, not just the motion at the top of the OoC.

The vibration pattern at the top of the OoC is depicted in Figure 1B as an almost-radial straight line. This simplified representation was used because of our ignorance of the real pattern of the motion of this vibrational mode. Most likely the motion has a complex pattern and includes transverse and longitudinal components. Further, we don't know how much of the OoC is involved in this vibrational mode (A vibrational mode is influenced by the geometry and material properties throughout the structure it is located in, but the main motion of the mode may be more restricted, e.g. the hypothesized TM radial-motion mode of Figure 1A). The top-of-OoC mode must extend to the RL to have the postulated effect on

OHC stereocilia deflection. Most likely this top-of-OoC vibration mode extends into the Hensen's cell region and includes what has been called the "lateral compartment" (Gao et al., 2014). The top-of-OoC vibration pattern may also extend as far down as near the bottom of the Deiters cells, based the extent of the "hot spot" described by Cooper et al. (2018), but we don't know the relationship of the "hot-spot" motion to the top-of-OoC vibrational mode.

The roles of specific anatomical features in the OoC vibrational modes are not known. The role, if any, of the TM in the top-of-OoC vibrational mode is unknown, but we note that "lateral compartment" motion was still present in mutant Tecta mice with no attached TM (Gao et al., 2014). The lateral compartment includes the Hensen's cells, a cell group with no known role in cochlear transduction. Perhaps the role of the Hensen's cells is to help form the resonant properties of the top-of-OoC mode and/or lateral-compartment mode. As shown in Figure 1B, the main axis of the top-of-OoC vibration pattern roughly parallels the fluid-filled spaces within the OoC, which raises the question of the role, if any, of these fluid spaces in the vibration pattern (Zagadou et al., 2020). Note that the outer tunnel (OT) is very small in histological sections, but in live tissue from the cochlear base, the OT is similar in size to the inner tunnel of Corti (Ramamoorthy et al., 2014; Cho et al., 2020).

A variety of evidence indicates that the top-of-OoC vibrational mode does not depend on OHC active processes to shape its vibration pattern or resonant frequency. Gao et al. (2014) found that the OoC "lateral compartment" has motion with a different phase than the rest of the OoC with a maximum amplitude at a frequency above the local BM CF, and that vibrations of the lateral compartment retained these properties even after death of the animal. This indicates that passive mechanical properties of the cochlea are responsible for the vibration pattern of the lateral compartment. Lee et al (2016) found that RL radial motion, when normalized by BM transverse motion, was tuned at a frequency above CF, and that this tuning and motion pattern remained after death, which shows that this RL radial motion is due to passive cochlear properties. As noted earlier, Dong and Olson (2013; 2016) concluded that the phase transition in the drive to OHCs is due to passive properties of the cochlea.

Although the observations cited above provide ample evidence that OoC motion is made up of a combination of different vibrational modes, it is not known whether the two modes shown in Figure 1 are the main ones that need to be considered or whether there are other important OoC vibrational modes. For instance, a traveling wave carried by the TM might be a third mode that needs to be considered. Finally, we note that the above analysis is based on data that are very sparse, and these conclusions should be considered as hypotheses. More-detailed data on OoC motion are needed.

Considering that RL motion has a different frequency response than BM motion, a recent study suggested that this is evidence for a "second filter" (Ramamoorthy et al., 2014). The term "second filter" implies that there is a cascade of filtering starting with the "first filter" of BM motion that provides the input to a second filter (Evans and Wilson, 1975). The term "second filter" is an oversimplification because the real cochlea is more complex with more than one traveling wave and with at least two vibrational modes that are concurrent, not sequential or cascaded.

3.2 Effects of OHC-motility at frequencies far below CF (tail frequencies)

We have focused on frequencies where there is cochlear amplification of BM motion, but at frequencies <0.7 CF, where the phase of TM-RL shear is not appropriate to cause cochlear amplification of BM motion, there is still BM motion leading to TM-RL shear, OHC stereocilia deflections, and expansions and contractions of OHCs. These OHC length changes can produce an order-of-magnitude amplification of RL movement that can extend many octaves below CF (Ren et al., 2016; He et al., 2018; Dewey et al., 2019; Fallah et al., 2019). Far below CF, the OHC length changes produce greater motion of the RL than of the BM because the BM is much stiffer than the RL (Mammano and Ashmore, 1993).

At low frequencies, when OHC motility produces large RL transverse motions, OHC motility also acts on the BM, but the effects of OHC motility on the BM at low frequencies has received inadequate attention in the literature. Low-frequency OHC motility should apply negative feedback and reduce BM motion (Fig. 2B, double-arrow-up vs. X) (Mountain, 1986). It had been concluded that BM motion at frequencies $\frac{1}{2}$ octave or more below CF is linear and unaffected by cochlear amplification (Robles and Ruggero, 2001). However, the effect of the negative feedback on BM motion from OHC-motility is expected to be small, almost linear, and not easily distinguishable from the normal variation from one measurement to the next. Nonetheless, there is evidence for expansive growth of BM motion at below-CF frequencies that is consistent with BM motion at low frequencies and low levels being reduced a small amount by negative feedback from OHC length changes (Ren and Nuttall, 2001; Rhode, 2007; Versteegh and van der Heijden, 2012). At frequencies considerably below the OHC membrane-time-constant corner frequency, the phase of OHC motility is 90° from the phase for producing positive or negative feedback (Fig. 2A, double-arrow-up vs. X). Thus, because the OHC force is out-of-phase with BM motion, and the BM is very stiff, OHC are expected to have little effect on the amplitude of BM motion at the lowest frequencies. Nonetheless, as noted earlier, OHCs could still have an effect on RL motion because the RL is much less stiff than the BM.

OHC motility produces small, local BM-traveling-wave amplifications that are accumulated as the traveling wave goes apically; here we consider whether a similar thing happens with RL amplification. RL motion is amplified relative to BM motion over a wide range of frequencies, extending from near the measuring-place CF to frequencies many octaves lower (Ren et al., 2016; He et al., 2018; Dewey et al., 2019; Fallah et al., 2019). The amplified RL motion in response to a probe tone can be suppressed by a low-level “suppressor” tone, but only if the suppressor tone is at a frequency near the measurement-point CF (Dewey et al., 2019; Fallah et al., 2019). For instance, at the 10 kHz place, the RL motion from a 5 kHz tone is amplified and can be suppressed by a low-level second tone, but the suppression is only for second-tone frequencies near the measurement-point CF of 10 kHz and when the second-tone response pushes the 10 kHz OHC MET channels into their nonlinear region. Second tones near 5 kHz or 20 kHz that produce a negligible motion response at the 10 kHz region would not change the 5 kHz response measured at the 10 kHz place, even though they could suppress RL motion at the 5 and 20 kHz places, respectively. This is consistent with the interpretation that RL motion is amplified by local OHC motility and that the increased RL motion is *not* carried apically in a RL traveling wave (Dewey et al., 2019; Fallah et al.,

2019). BM traveling-wave motion is amplified and RL motion is amplified relative to BM motion, so RL motion in response to frequencies near the local CF receives both BM cochlear amplification and local RL amplification, while RL motion in response to tones much lower in frequency than CF (tail-frequency tones) receives only local RL amplification. Exactly how far along the cochlea from CF local RL amplification extends is difficult to determine because the RL amplification region is explored by adding a suppressor tone whose response is not punctate along the cochlea. A comparison of the spread of second-tone suppression of BM and RL responses to CF tones, indicated that BM cochlear amplification extends more basally than RL amplification (Dewey et al., 2019; Fallah et al., 2019), which is consistent with RL amplification not spreading much along the cochlear length.

Siegel (2018) hypothesized that stimulus-frequency-otoacoustic-emission (SFOAE) components arise far basal of the SFOAE CF region due to basal RL amplification. Evidence against far-basal RL amplification producing SFOAE components when there is no far-basal suppressor tone comes from cochlear perfusion experiments. As an OHC blocking agent was perfused from the apex to the base, it removed all of the SFOAE produced by a low-to-mid-frequency tone by the time the solution had reached the region two octaves basal of the SFOAE-tone's CF region (Goodman et al., 2020). If regions more than two octaves basal to the SFOAE tone's CF region were producing SFOAE components, reduction of these SFOAE components would be seen as the solution went through these regions. However, this was not observed, which implies that frequency regions far basal to the SFOAE CF region do not produce SFOAE components *when there is no suppressor tone at these far-basal high frequencies*. In these basal regions there would have been RL amplification at the below-CF SFOAE frequency, but the resulting RL-frequency amplified RL motion was not creating an SFOAE-frequency emission. The Goodman et al. (2020) results indicate that amplified RL motion far basal of the SFOAE CF region is not coupled to the backward traveling wave that produces SFOAEs, which is consistent with the conclusion that amplified RL motion doesn't contribute to a forward traveling wave (Dewey et al., 2019).

RL motion at tail frequencies can be expected to influence inner-hair-cell (IHC) stereocilia deflections. Such an influence is demonstrated by the reduction of tail-frequency auditory-nerve-fiber responses produced by excitation of medial efferents (Stankovic and Guinan, 1999). This is explained by activation of medial efferents reducing OHC motility and thereby reducing RL motion and the excitation of IHC stereocilia and auditory-nerve fibers. Another demonstration that OHC motility affects tail-frequency auditory-nerve responses is that high-level, very-low-frequency, "bias" tones that drive OHC stereocilia into MET saturation, can reduce tail-frequency auditory-nerve responses (Nam and Guinan, 2018).

3.3 The Phase of Reticular Lamina Transverse Displacement

One aspect of cochlear motion that is not well understood is the phase of RL transverse motion relative to BM motion. In most measurements, RL and BM motion had approximately opposite phases at low frequencies and as frequency was increased there was an increasing lag in RL motion re BM motion so that near CF their phases were approximately equal (Ren et al., 2016; He et al., 2018; Dewey et al., 2018). However, Dewey

et al. (2019) found an almost opposite pattern. To help understand RL transverse motion, we calculated RL *transverse* displacement for two possible phases of RL *radial* motion advance re BM motion (130° and 180°) and three amplifications of RL motion relative to BM motion (1, 2 and 10), as indicated by the key in the upper right of Figure 2. We used a 130° RL-radial-motion phase advance because Dong and Olson (2013) found that the ST voltage (a proxy for OHC current) led BM displacement by $\sim 130^\circ$ in the frequency region ~ 0.7 CF to CF. Presuming that the TM radial phase change is negligible, this means that the RL radial phase controlled the OHC stereocilia deflection and had a 130° phase advance re BM motion. For comparison we also show a 180° change, which is the phase change found for lateral compartment motion (Gao et al. 2014) and also represents the change expected from the TM resonance model. These are shown in Fig. 2C by the change from row 6, BM displacement, to the row 5 lines (solid for 180° , dashed for 130°) – note that the phase of RL radial displacement is not shown as a separate row because it is the same as the phase of OHC stereocilia deflection in row 5. The amplitude of measured RL transverse motion relative to BM motion varied with frequency relative to CF, and was typically largest for frequencies far below CF, smallest at CF, and near unity in a dead animal; we approximated these with values of RLgain = 10, 2 and 1, where RLgain is defined as the amplitude of RL transverse motion relative to BM motion. RL transverse motion is produced by BM motion boosted by OHC motility. Considering this, RL transverse-displacement phase was calculated by adding (i) the BM displacement at the phase shown in Figure 2, row 6, to (ii) a motion at the phase of the OHC push upward, as shown by the motion of the OHC top (Fig 2, row 2), multiplied by a factor that makes the net RL amplitude (i.e. BM amplitude plus the extra from OHC top push) be the RLgain value times the BM displacement amplitude (for RLgain = 1, this factor is zero). The resulting waveform, normalized to have the same plot amplitude as BM motion, is shown in Figure 2, row 1.

The calculated RL transverse displacement can be followed across frequency regions by comparing the phase delays of the circles in Figure 2, row 1, across columns A-C (in C, the circle is on the 130° waveform, but the other waveforms are also possibilities). As frequency increases, the RL transverse phase is first delayed 90° because of the OHC membrane time constant (column A to B) and then is advanced ($\sim 135^\circ$ to $\sim 225^\circ$) depending on the combination of RL radial phase advance and the RLgain. These calculated results show the expected change in the phase of RL transverse motion can be very large, which is also true for the measured changes in the phase of RL transverse motion. Nonetheless, the RL phase changes shown in Figure 2, row 1, do not correspond well to any of the reported RL phase changes.

There are many possible explanations for the differences between the calculated and reported RL transverse phases, as well as for the differences in RL phase change across reports. In our analysis, we assumed all motion was within a transverse plane. In the experiments, the viewing angles differed across experiments and were not always well specified. Different viewing angles would get a different mix of radial, transverse and longitudinal motion (see section 3.4), which means that when reporting OCT measurements it is important to specify the viewing angle relative to all three dimensions of the OoC place being measured. In addition, the spatial extent of the OoC regions sampled by the OCT measurements and called “RL” or “BM” might be different across experiments because of

the different viewing angles, the different resolutions in the OCT systems, and/or because the longitudinal alignment BM and RL measurement positions varied across experiments. More information is needed about many aspects of cochlear mechanics before RL phase is understood.

3.4 Limitations of the above analysis

In the above analysis we have assumed that Deiters cells are rigid so that the force applied by the OHCs on Deiters cells is directly transmitted to the BM, however Deiters cells may not be rigid. If Deiters cells provide a flexible or viscoelastic coupling between OHC force and BM motion, this would introduce another phase change that would need to be accounted for in the analysis of the OHC stereocilia phase needed for cochlear amplification. The data that are most suggestive that Deiters cells are not rigid comes from Cooper et al. (2018) who found an OoC “hot spot” that showed large motion responses extending from the RL to the Deiters-cell region $\sim 20\mu$ from the BM. They also found that one end of the Deiters cells moved twice as much as the other end. One interpretation of these data is that the Deiters cells are flexible, especially at $\sim 20\mu$ from the BM. But, as pointed out by Cooper et al. (2018), the measurements only show motion that is along the OCT optical axis, so the measurements don’t capture all of the motion. When Cooper et al. (2018) changed the optical axis from pointing apically to pointing basally, this reversed the direction of the phase change of the hot-spot motion relative to BM motion, which indicates that a large fraction of the hot-spot motion is in the longitudinal direction. These measurements are consistent with the Deiters cells rotating in the longitudinal direction about a spot near the BM (Cooper et al., 2018). Whether the axis of rotation is at the BM, or $\sim 20\mu$ from the BM, was not well determined. The dramatic increase in Deiters cell motion approximately $\sim 20\mu$ from the BM may not be because the Deiters cells are flexible at that spot, but because this is the location where the measured component of longitudinal motion outweighs the measured component of transverse motion. Note also that if a rigid rod rotates about a spot $1/3$ of the way from the end, one end will move twice as much as the other end, which shows that differences in motion from one end to the other of a structure are not compelling evidence that the structure is flexible, especially when the motion is measured along only one dimension.

While the flexibility of the Deiters cells remains uncertain, the data clearly show that there is a large component of the motion in the longitudinal direction. Motion in the longitudinal direction that is similar in magnitude to transverse or radial motion has been found previously (Karavitaki and Mountain, 2007) and is shown by models that include a representation of the complex OoC geometry in which OHCs tilt basally and the Deiters cell phalangeal process extends apically (Motallebzadeh et al. 2018). Perhaps the bigger omission in our earlier analysis is the lack of considering motion in the longitudinal direction, not the lack of possible flexibility in Deiters cells (both are likely to be important). From the earliest cochlear models, with a few exceptions (e.g. feed-forward models such as Motallebzadeh et al. 2018), it has been thought to be adequate to represent OoC mechanics with the motion considered to be in a single plane. Cooper et al’s (2018) measurements show that is necessary to consider OoC motion in all three directions, including motion that extends out of a single transverse OoC plane. Indeed, it may not be possible to understand

cochlear amplification without including motion that goes beyond a single transverse plane (Motallebzadeh et al. 2018).

Overall, the results (particularly, Cooper et al., 2018) point to the need for more measurements in which the viewing angle is changed so that the motion can be measured in two, or ideally three, dimensions. In addition, experimental papers should provide information about the viewing angle(s) and discuss how the motions of different structures included in a voxel's tissue volume may affect the results, especially when the viewing angle has been changed which often changes what was included in a voxel. The need for such additional information is pointed out by the opposite results obtained on the phase of RL motion from what seems like similar measurements when assessed without this crucial additional information.

Our discussion has focused on frequency regions *above* the corner-frequency of the OHC-membrane low-pass filter. However, at frequencies substantially *below* the OHC membrane cutoff, OHC transmembrane voltage is in phase with OHC current, so that for low-CF regions, somatic motility is in phase with OHC-stereocilia deflections, and the OHC stereocilia phase to produce cochlear amplification is different by 90° compared to the phase at high frequencies. This difference may be an important contributor to the different cochlear behavior above versus below the cochlear “apical-basal” transition region (Shera and Guinan, 2003; Shera et al., 2010; Moleti et al., 2017; Dong et al. 2018; Christensen et al., 2018). It remains to be seen what role having the sound frequency be below the OHC corner frequency has in the following low-CF behavior: (1) low-CF mechanical tuning is low-pass, whereas high-CF tuning is band pass with V-shaped tips, (2) low-CF mechanical tuning becomes sharper as sound level increases, and (3) low-CF group delays can increase with increasing sound level (Recio-Spinoso, and Oghalai, 2017; 2018).

Finally, there are several phenomena whose roles in cochlear amplification are not understood but might be important: (a) OHC stereocilia motility (Jia and He, 2005), (b) TM electrokinetic forces (Sellon et al. 2018), and (c) TM movement near the OHC stereocilia might be poorly coupled to the tops of the OHC stereocilia so that there is a phase difference between TM and OHC-stereocilia motions (Sellon et al. 2018).

3.5 Insights from models

Hubbard (1993) showed that a cochlear model with two interacting traveling waves could produce BM tuning curves with realistic amplitude and phase curves, but he did not identify a physical structure that carried the second traveling wave. With the discovery of longitudinal traveling waves of radial motion in the TM, the second traveling wave has been suggested to be a TM traveling wave (Ghaffari et al., 2007). Although the relationship between the traveling waves in the excised TMs of Ghaffari et al. (2007) and those in live mice is unclear, measurements in intact mouse cochleae show that the TM has traveling waves that are different from BM traveling waves (Lee et al. 2015). Whether the top-of-OoC mode is also a separate traveling wave, or is so strongly coupled with the BM traveling wave that it should *not* be considered as separate, is an unanswered question. However, it seems clear that there are at least two interacting traveling waves in the cochlea.

The interaction of different traveling waves may be a key factor in achieving the correct phase of OHC stereocilia to achieve cochlear amplification. Some recent models have used simple structural elements based on the BM and the TM, with varying degrees of coupling between them, and found that the vibrational pattern showed two modes of motion. With proper coupling between the BM and TM elements, these models could produce two separate, but interacting, traveling waves (Lamb and Chadwick, 2011; 2014; Cormack et al., 2015). At a given cochlear place, the mixtures of these modes on the BM and the TM varied with frequency. In a model that allowed differential radial motion across OoC structures, there were transitions in which mode was dominant and these transitions produced changes in phase across frequency of the kind needed to bring about cochlear amplification (Lamb and Chadwick, 2011; 2014). These models show that cochleae with relatively simple geometries and two interacting modes can produce transitions between modes that are similar to those that appear to be present in real cochleae. These models also point to the important role of the coupling between the interacting modes. The main coupling between the OoC and the TM is through the OHC stereocilia. Perhaps OHC stereocilia have “W” shapes (which may provide stiffness like sheet-metal corrugations) to provide increased OoC to TM coupling in both radial and longitudinal directions. To understand the roles of vibrational modes and traveling waves in cochlear amplification, as well as what causes them to change in the critical frequency region below CF, we need both more detailed motion measurements and more detailed models. There is still a lot to learn about cochlear amplification.

4. Conclusions

Although the TM-resonance hypothesis is no longer tenable, the original insight that led to this hypothesis is still correct: to get cochlear amplification, the pattern of vibration in the TM versus in the RL must be different than the vibration predicted by the Davis (1965) model. Based on present data, it appears that the correct phase is produced by a change in vibrational modes that involve the whole OoC, and not just by a simple TM resonance.

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Highlights

- Cochlear mechanical motions that deflect outer-hair-cell stereocilia are reviewed
- Recent motion measurements eliminate a proposed tectorial-membrane resonance
- A reticular lamina phase advance produces the phase for cochlear amplification
- A change in the dominant organ of Corti vibrational mode yields the phase advance
- Poorly understood areas of cochlear mechanics are reviewed and considered

Fig. 1.

Representation of the organ of Corti (OoC) and suggested vibrational modes. **A:** A 1980's conception of vibrational modes showing a mode due to OoC rotation about the foot of the inner pillar cells (IP) (blue curved line with arrows), and a mode due to near-radial motion of the tectorial membrane (TM) (brown straight line with arrows). The TM vibration was thought to have a resonant frequency $\frac{1}{2}$ octave below the characteristic frequency (CF) of basilar-membrane (BM) motion, so that at CF, TM motion would *lag* BM motion by $\sim 180^\circ$.

B: A current conception of OoC modes showing the same OoC rotational mode and a vibrational mode of the top of the OoC (long brown straight line with arrows). At frequencies 0.7 to 1 times CF, the top-of-OoC vibrational mode produces radial motion of the reticular lamina (RL) that *leads* TM and BM motion, thereby deflecting OHC stereocilia in the correct phase to produce cochlear amplification. This mode is depicted as a straight-line, but we don't know the actual direction or spatial extent of its vibration pattern. This mode probably includes transverse-motion and longitudinal-motion components. In the OoC representations, clear areas are fluid filled, shaded areas indicate regions with tissue, and areas that have been thought to have important structural roles are dark-green. The fluid-filled regions from the inner tunnel of Corti (IT) to the outer tunnel (OT) are connected by fluid-filled spaces between the outer-pillar cells and fluid spaces around the outer hair cells (OHCs); these fluid connections are not shown in the drawings. IHC=inner hair cell, S=sulcus, DC=Dieters cells, HC=Hensen's cell region.

Fig. 2.

Response waveforms showing relative phases at one location along the cochlea for three frequency regions at which the response phases are expected to differ (columns, labeled at top) and various physiologic variables (rows, labeled at right and numbered from the top, but note that the sequence of causality goes more from the bottom to the top). **A:** The lowest frequency region where the sound frequency (F) is substantially *below* the outer hair cell (OHC) membrane corner frequency, and the organ of Corti (OoC), including the tectorial membrane (TM), does not have resonant motion (labeled as “firm”). **B:** A middle frequency where F is substantially *above* the OHC corner frequency and the OoC is “firm”. **C:** the highest frequency, within $\frac{1}{2}$ octave of the characteristic frequency (CF), a frequency region that is substantially *above* the OHC corner frequency and where a resonance (either from the TM or an OoC vibrational mode) substantially changes the phase of the deflection of OHC stereocilia. In each row, symbols indicate similar points of phase. RL displacement phase (row 1) was calculated for RL amplitudes that are 1, 2 or 10 times the BM amplitude as indicated by the row-1 key at the upper right (in column A, 2 and 10 yield the same phase). In column C, rows 1–5, results are shown for RL-radial-motion phase changes of 130° (dashed lines) and 180° (solid lines). Row 5 (labeled: OHC stereocilia deflection) also shows the phase of RL-radial-motion. See text for further explanation.