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The Functional Contributions of Links in Mammalian Cochlear Hair Bundles

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Abstract. In the mammalian cochlea, hair bundles of the sensory outer and inner hair cells detect mechanical signals. A hair bundle comprises a set of rod-like stereocilia that pivot around their insertion points in the hair-cell's apex. Stereocilia are linked by gating springs and connectors, also known as top or shaft connectors, side, lateral, or ankle links. Gating springs link neighboring stereocilia of differing height, while connectors link all neighboring stereocilia. Sound-induced gating-spring oscillations open and close mechanoelectrical transduction channels attached to the gating springs, causing oscillations in the hair cell's receptor current. In contrast to gating springs, connectors are not attached to channels and their functional role is unclear. To determine how the specific properties of gating springs and connectors contribute to outer-hair-cell bundle function, we use a computational model of an outer-hair-cell bundle, which accounts for nonlinear hair-bundle splaying at rest, nonlinear fluid forces on stereocilia, and nonlinear channel gating. The model is validated by reproducing many experimental observations, including stereocilium splaying at rest and hair-bundle stiffness decreases caused by breaking gating springs or connectors. We discuss how varying the gating-spring and connector stiffnesses affects the receptor current in response to stimulation at the characteristic frequency of the hair cell.

INTRODUCTION

In the auditory, vestibular, and lateral-line organs of vertebrates, hair bundles convert forces induced by sound, head motion or position, and fluid into receptor currents [1]. A hair bundle comprises rows and columns of rod-like stereocilia protruding from the hair-cell's apex and increasing in height toward one edge of the bundle. In a column, stereocilia of increasing height are linked by gating springs, which transmit forces that gate (open and close) mechanoelectrical transduction (MET) channels. Rows are formed by stereocilia of similar height, which are not linked by gating springs. Deflection of a hair bundle toward its tallest row (row 1) causes its stereocilia to pivot, gating-spring forces to increase, and MET channels to open. Gating springs comprise tip-links, made of the proteins protocadherin 15 (PCDH15) and cadherin 23 (CDH23), and other elastic elements in series with the MET channels [1, 2]. Stereocilia are also linked within rows and columns by connectors, which do not gate channels. It has been proposed that connectors increase the coherence of stereociliary motions and decrease hair-bundle damping, but recent work in outer-hair-cell (OHC) bundles show that connectors can decrease the uniformity of stereociliary motions and increase hair-bundle damping, calling the role of OHC connectors into question [3, 4, 5]. Connectors comprise different elements in different types of bundles and include top and shaft connectors, side, lateral, and ankle links [6, 7, 8, 9, 10].

The structure and function of hair bundles differ between species, between organs, and between different locations within an organ, but we have a limited understanding of the relationship between a hair bundle's structure and its function [1]. Gating-spring and connector stiffnesses differ between different types of bundles and between different locations within an organ, but we do not know how specific gating-spring or connector stiffnesses contribute to bundle function in the mammalian cochlea [3, 11, 12, 13]. In addition, connector stiffness may differ between rows and columns within the same bundle and we have no information about gating-spring or connector damping [12]. Here we focus on the contribution of gating-spring and connector stiffness to OHC-bundle function in the mammalian cochlea.

The function of an OHC bundle is to convert sound-induced forces into receptor currents. OHC structure changes from the cochlear base to apex: bundle stiffness decreases, stereocilium number decreases, stereocilium height increases, pivot and gating-spring stiffness decrease, and gating-spring resting tension decreases [11, 14, 15, 16, 17]. OHC connectors comprise stereocilin, immunolabeling of which decreases in intensity from base to apex, suggesting a decrease in connector stiffness and damping from base to apex [9]. Because OHC mechanics changes with characteristic place along the cochlea, we study OHC bundles corresponding to a specific characteristic-frequency (CF) region (4 kHz). Using a computational model, we determine the OHC bundle receptor current in response to

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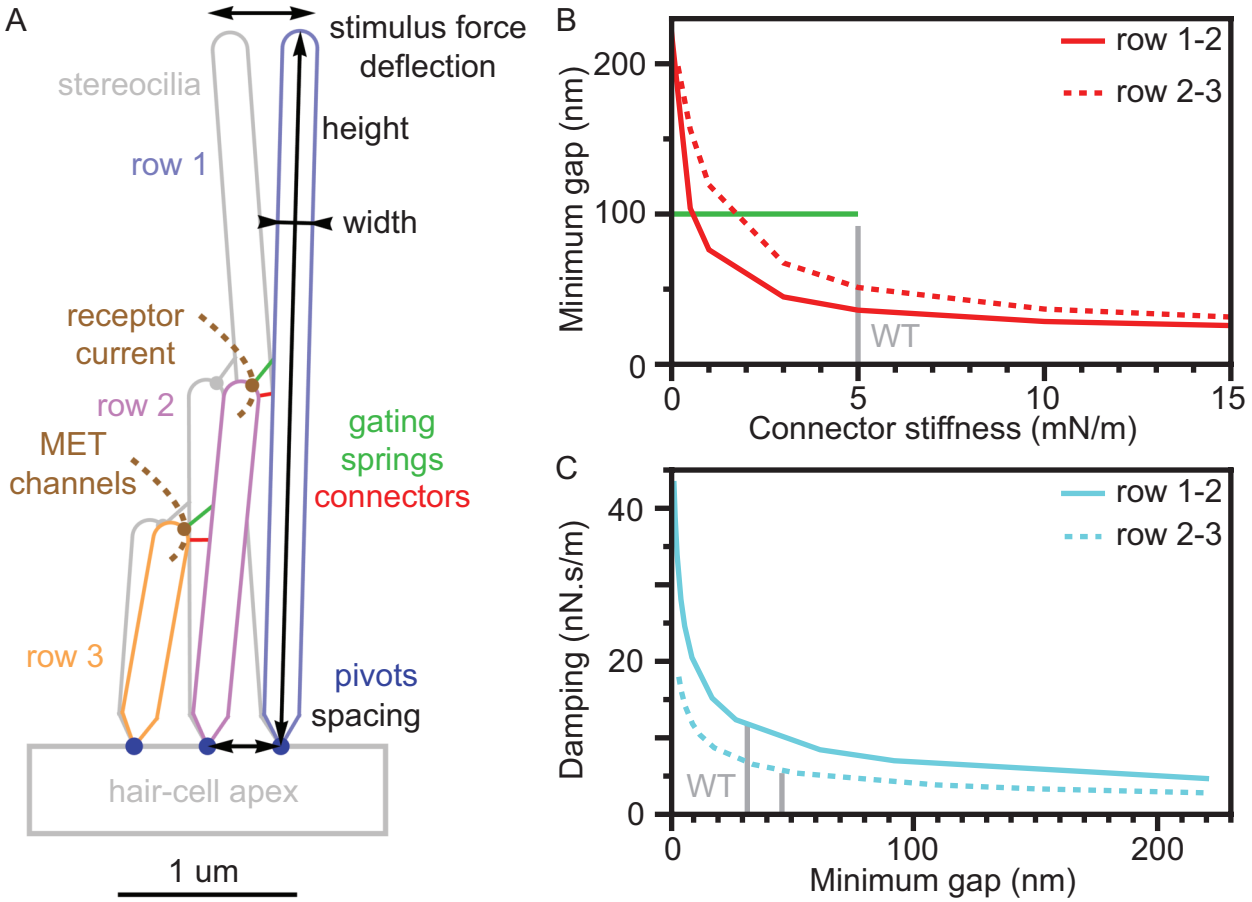

FIGURE 1. (A) An OHC bundle column. Stereocilia of different rows have different heights, widths, and pivot spacing (black). In response to a sound-induced stimulus force, stereocilia deflect around their pivots (blue) in the hair-cell apex. Stereocilia are linked by connectors (red) and gating springs (green) that gate MET channels (brown circles) through which the receptor current (brown dashed lines) flows. To help visualize the deflection, a deflection much larger than the physiological maximum (less than a stereocilium's radius) is shown. (B) In a bundle without gating-springs, the minimum gaps between neighboring stereocilia are shown versus the connector stiffness. The minimum gap is along the undeflected connectors in panel A. The wild-type (WT) connector stiffness is indicated by the vertical gray line. The length of a reforming gating spring composed of PCDH15-PCDH15 is indicated by the horizontal green line. (C) Fluid damping coefficients associated with changing stereocilium separations along the connectors are shown versus the minimum gap between stereocilia. The WT values of the minimum gaps are indicated by vertical gray lines.

oscillatory tectorial-membrane forces versus the stimulus frequency. To determine the contributions of gating-springs and connectors, we vary the properties of these components and calculate the changes in the receptor current.

OUTER HAIR CELL BUNDLE MECHANICS

To demonstrate some of the consequences of varying gating-spring and connector stiffness, we use an OHC bundle model consisting of a single column of stereocilia (Fig. 1A). Because the stereocilia pivot rather than bend, a single connector between neighboring stereocilia is sufficient to capture the connector's mechanical effects [18, 19, 20, 21]. Pivots, gating-springs, and connectors are viscoelastic elements. Fluid between neighboring stereocilia causes damping and couples the stereocilia [3, 22, 23]. We describe each MET channel using two-state kinetics [24]. Myosin motors set the gating-spring resting tensions and the resting open probabilities [1, 11, 25, 26, 27]. We do not include adaptation for three reasons [1, 26]. First, adaptation's mechanism in OHCs remains a matter of debate [27, 28, 29]. Second, adaptation has a small effect on OHC-bundle mechanics [27, 29]. Third, by omitting adaptation, we distin-

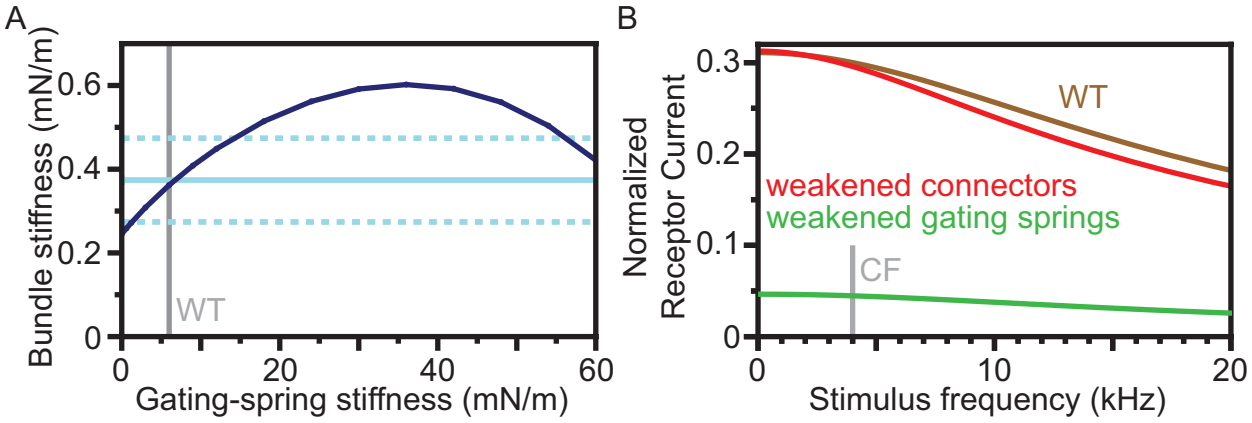

FIGURE 2. (A) The bundle's stiffness is shown versus the gating-spring stiffness (dark blue). For comparison, the experimentally-measured hair-bundle stiffness is shown as horizontal light-blue lines (mean (solid) ± standard deviation (dashed)) [11]. For a single column, this stiffness is the measured bundle stiffness divided by the number of columns in the bundle. The WT gating-spring stiffness (gray line) is chosen such that the model bundle's stiffness matches that of experiments. (B) The normalized receptor current is shown versus the stimulus frequency for the WT bundle (brown). Receptor currents for weakened (10% of the WT values) gating-springs (green) or connectors (red) are also shown. A vertical gray line indicates the bundle's CF.

guish its effect on the receptor current from the viscoelastic effects we discuss here. Based on prior measurements, we expect adaptation to high-pass filter the receptor current with a corner frequency of about 600-800 Hz for a 4 kHz CF OHC [30, 31, 32]. The tectorial membrane displaces the OHC bundle and the fluid around the bundle almost simultaneously, thus there is little fluid damping caused by the surrounding fluid on an OHC *in situ* [33, 34]. We stimulate the row 1 tip of the OHC bundle using sinusoidal tectorial-membrane forces. The receptor current is the sum of the MET currents through each channel and is normalized to the maximum current for fully open channels.

Wild-type (WT) OHC bundle parameters correspond to the 4 kHz CF region in the rat and mouse. We determine the stereocilium heights, widths, pivot spacings and resting gating-spring lengths from published electron and light microscopy data [11, 35, 36]. We validate the model by reproducing many different experimental observations. For example, by matching the bundle's stiffness and damping to that of experimental measurements, we determine the pivot, gating-spring, and connector stiffness and damping [4, 11]. We describe channel dynamics using a recent estimate of the channel time constant and a gating swing such that the width of the receptor-current activation curve matches experimental measurements [24, 29, 37]. By integrating analytical expressions for the damping between separating and sliding cylinders, we calculate the fluid damping between stereocilia and validate these calculations by comparing them with prior computational fluid dynamics results [3, 23, 38, 39].

RESULTS

The connectors and gating springs cause the stereocilia to lean toward each other at rest (gray stereocilia, Fig. 1A). The leaning angles and the gaps between neighboring stereocilia depend nonlinearly on the link stiffnesses (e.g. Fig. 1B). Noise breaks OHC tip links, the extracellular parts of gating springs, increasing the gaps between neighbors [2, 40, 41, 42]. In other words, noise causes stereocilia to splay in their resting state. Tip links can reform, first as 100-nm PCDH15-PCDH15 filaments before they are replaced by PCDH15-CDH23 filaments [2, 43]. Without connectors, however, the minimum gaps between neighboring stereocilia would be too large (> 200 nm) to allow the tip links to reform. In our model, increasing the connector stiffness decreases the minimum gaps between neighbors, which would allow the tip links to reform (Fig. 1B). As suggested previously, the WT connector stiffness is sufficiently large to constrain the minimum gaps and limit stereocilium splaying at rest [9, 44].

Fluid damping between neighboring stereocilia depends nonlinearly on the gaps between the stereocilia [3, 22, 23]. In our model, the fluid damping coefficient associated with stereocilia separating decreases as the minimum gap between stereocilia increases (Fig. 1C). Thus, decreasing the gating-spring or connector stiffness decreases the fluid damping between neighbors. Because the row 2-3 gap exceeds that of row 1-2 and row 3 is shorter than row 2, the row 2-3 damping coefficient is less than that of row 1-2.

Owing to MET channel gating, hair-bundle stiffness depends nonlinearly on gating-spring stiffness (Fig. 2A). This nonlinearity arises because hair-bundle stiffness depends on the gating-spring stiffness and the MET channel's open probability, which depends nonlinearly on the gating-spring stiffness [13, 24, 26, 45]. At all gating-spring stiffness values, MET channel opening decreases bundle stiffness, a phenomenon known as gating compliance [13, 45]. Although bundle stiffness initially increases with gating-spring stiffness, gating compliance also increases with gating-spring stiffness, causing bundle stiffness to eventually decrease with gating-spring stiffness. For the WT bundle, decreasing gating-spring stiffness decreases bundle stiffness, in agreement with experimental observations [11].

Although the bundle displaces less in response to stimulus forces (bundle stiffness increases) as the gating-spring stiffness increases, the CF receptor current in response to an oscillatory stimulus force increases markedly with gating-spring stiffness (Fig. 2B). In contrast, the CF receptor current increases very little with increasing connector stiffness. Because of bundle damping and MET channel kinetics, the receptor current decreases with increasing stimulus frequency.

DISCUSSION AND CONCLUSION

Although we model only a single column of an OHC bundle, we gain insight into three nonlinear contributions of gating springs and connectors to OHC bundle function. First, connectors constrain the gaps between neighboring stereocilia, allowing broken gating springs to reform. Second, gating-springs and connectors regulate the damping between neighbors by constraining the gaps between them. Third, gating springs can increase or decrease bundle stiffness, because they also regulate MET-channel gating. Additionally, we find that the receptor current is much more sensitive to gating-spring than connector stiffness.

The increase in gaps between neighboring stereocilia with decreasing connector stiffness agrees with the increase in splaying at rest seen in OHC mutants lacking connectors [4, 9, 10]. Connectors may also protect gating springs from breaking in response to high stimulus intensities, but we expect this protection to come at a cost [44]. If connectors act in parallel with gating springs and reduce gating-spring forces at high stimulus intensities, then they will also reduce gating-spring forces at low stimulus intensities, decreasing bundle sensitivity. Whether connectors increase OHC bundle coherence remains to be determined [3]. We also show that connectors regulate bundle damping and have little effect on CF receptor currents.

Bundle stiffness varies nonlinearly with gating-spring stiffness. The size of this effect increases with the gating swing size, the change in the gating-spring's length when the MET channel opens [1, 26, 45]. Here we use a gating-swing of 0.5 nm to match the measured width of the current activation curves [29]. It is likely, however, that the activation curve width is overestimated and that we have consequently underestimated the gating-swing size [12, 28, 29]. Thus, the gating-spring stiffness at which the bundle stiffness peaks may be smaller than we show here. Because of the experimentally-observed increase in gating-spring stiffness toward the cochlear base, gating compliance may contribute more to the stiffness of basal bundles than apical bundles [11, 14].

The contributions of gating-spring and connector damping remain to be determined. We find that links regulate fluid damping and prior work shows that MET channel gating dynamics causes damping [24]. It is difficult to disentangle these contributions to bundle damping from intrinsic link damping, even though we have data showing that bundle damping changes when links are broken [4]. We expect link damping to decrease bundle displacements and receptor currents and because mammalian hair bundles must respond to high stimulus frequencies, we expect link damping to be small. Links may, however, have unavoidable damping owing to physical constraints on their biomolecular structure. Link damping may contribute to calcium-independent adaptation or the coherence of stereociliary motions at high stimulus frequencies [3, 46].

Because of the minimal number of assumptions and because the single-column model is validated by matching experimental observations, we expect our single-column 2D results to apply to 3D OHC bundles with adaptation. However, we also expect the results to be modified in 3D bundles with adaptation and for these bundles to exhibit additional effects associated with gating-spring and connector mechanics. Our single-column model does not account for the possibility that OHC columns do not move in parallel *in vitro*, that there are connectors between columns, and that adaptation affects the receptor current in response to low-frequency stimuli [5, 7, 8, 9, 10, 30, 47]. Comparing 2D bundles, 3D bundles, and bundles with and without adaptation will further clarify the contributions of gating-springs and connectors to OHC function.

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COMMENTS AND QUESTIONS

Karl Grosh: Nice talk. I wonder if you could just clarify the twenty degree phase angle and how that changes with frequency and what are the contributions and mechanisms that give rise to it. I thought that was really interesting.
Author: It's coming predominantly from the channel dynamics. It takes time for the channels to open. This then creates an effective corner frequency. If we make the time constant smaller, we can raise the corner frequency.
Karl Grosh: Is this a purely linear effect?
Author: Purely linear. Everything I've shown you is linear response. We linearize all of the nonlinear equations of motion.
Ernst Dalhoff: So if I understand correctly this is a cycle-by-cycle process. Even if the whole system adapts, I always would expect these twenty degrees?
Author: Yes, you will always get a phase difference, although its exact magnitude depends on the the bundle's properties. This is completely passive. I have deliberatively avoided any active process.

Robert Raphael: It seems there is an assumption that you are making that the open probability and rate constants of the mechanotransduction channel are the same in each bundle. Experimentalists tell us they can't measure this. So you could have the possibility of different characteristics of the channels.
Author: Absolutely. We're assuming that all the channels within a single bundle have the same properties. You are correct that as you look at bundles tonotopically, we know that the conductance of these channels changes. I've shown you that the gating-spring stiffness changes. In fact, the gating-spring stiffness contributes to the open probability curve I showed you. As you increase the gating-spring stiffness, this curve is going to get sharper. So in fact channel opening is changing as you increase the gating-spring stiffness.

Justin Faber: Thank you for the very nice talk. I have two questions. This elliptical or circular motion, do you think there could be any fluid-dynamical advantage of avoiding friction or is this way too small of a scale?
Author: Any time you see elliptical motion, it's indicating dissipation. I think the system is not optimized. Optimally, all of the columns would be parallel. It's just a limitation of development.
Justin Faber: I'm wondering, why three rows of stereocilia instead of two or ten?
Author: I'm wondering that two. One of the things we're working on is taking this type of model and changing the number of rows, to try and determine how changing the number of rows contributes. That's one of the mysteries. Why are mammalian cochlear hair bundles special with three rows? Nonmammalian hearing bundles have 11-12 rows of stereocilia.

Marcel van der Heijden: Very, very beautiful talk. Are you aware that recent OCT data show distinct DC or rectified responses of outer-hair-cells? What are the implications of your findings for that?
Author: It's something we're working on. We're aiming to also calculate the DC response, but we notice that the AC response is always at the start bigger than the DC response and that there's a trade-off. The question is what's more salient for encoding the signal.
Marcel van der Heijden: And maybe related to it: directional sensitivity. The way the bundles are stimulated may very well depend on frequency and SPL in vivo.
Author: The wavelength of the traveling wave is much larger than the size of the hair bundle, so there's no reason to expect that there wouldn't be anything but uniform stimulation. However, I can't rule out that there's not some micromechanical structure that's determining a non-radial stimulus on the hair bundles. All we can do is push the bundle in the preferred direction and change the angle of stimulation and look at the response.

Jonathan Ashmore: What happens when you have really stubby little stereocilia, as you find in the super high-

frequency animals like bats and things like that?

Author: Then the effective stiffness of the stereocilia is very, very high because the effective stiffness goes like one over the length squared. Very handwavingly, you're increasing the corner frequency of the system because you are increasing the stiffness. The problem however is, as you decrease the subtectorial space size, you increase the drag. It's weird to me that you're increasing the drag as you go to high frequencies. It's not just the drag on the hair bundle you're fighting. You're also fighting the drag in the subtectorial space.

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