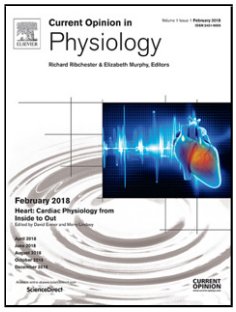

Tonotopy of cochlear hair cell biophysics (excl. mechanotransduction)

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Current Opinion in Physiology: Mammalian Hearing

Tonotopy of cochlear hair cell biophysics (excl. mechanotransduction)

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Abstract

The cochlea is tonotopically organised to ensure that the auditory nerve fibres can be frequency coded in an orderly manner. In part the mechanism depends on the structural and mechanical organisation of the cochlea but it also requires that the individual cells have an organised expression of ionic channels in the basolateral membrane. This short review will discuss evidence for varying gradient distributions of K^+ channels along the cochlea in both mammalian and non-mammalian hearing organs. It will also describe how the gradients are set up and address the question of whether OHCs contribute uniformly to mammalian cochlear tuning.

Keywords: cochlea; ion channels; outer hair cells; inner hair cells; tonotopic gradient; potassium channel; prestin

Introduction

The main function of the cochlea is to separate out different frequencies from a complex sound. Without this capability we should not be able to make sense of an acoustic environment. To this end the cochlea is organised as a one-dimensional array of analysers arranged so that each frequency is mapped onto a specific place in an orderly manner. A tonotopic map is found in both vertebrates as well as in some invertebrates. The map depends on the distribution of some form of resonance, mechanical or electrical, where the maximal response to a particular sound frequency is organised monotonically along the length of the cochlea. In vertebrates, such resonances can arise from the properties of individual hair cells, through either the mechanics of the hair bundle, an intrinsic electrical resonance of the cell membrane or, in the case of the mammalian cochlea, from an enhanced mechanics of the cochlear partition.

Which biophysical parameters of a hair cell are critical for a tonotopic map? In most non-mammalian systems where the upper hearing limit lies below 10 kHz, whole cell patch clamp recording has revealed specific ionic mechanisms in the basolateral membrane of the hair cells: the membrane properties are dominated by K^+ and Ca^{2+} currents [1] [2] [3]. It has been known since the earliest hair cell recordings that the interplay between these two currents leads to an electrical resonant behaviour where the membrane potential can oscillate at a preferred frequency. The

interaction between large conductance K^+ (BK(Ca)/KCNMA1) channels and voltage gated Ca^{2+} channels (VGCC), mainly Cav1.3 homologs, produces a damped oscillatory response when current is injected through the apical transducer channels. Such mechanisms are known to occur the auditory organs of amphibia, reptiles and birds (reviewed in [4]) and the tonotopic distribution arises from varying the gating kinetics, the density and the co-localisation of the channel types. A number of alternatively spliced isoforms of the KCNMA1 gene, both in turtles and in chicks, are known to underpin this variation but it remains unclear which gene networks set the global tonotopic expression, even though some determinants of the axis are set up early in development (e.g. [5].) The investigation of these accessible non-mammalian systems has been the groundwork for the study for mammalian systems.

Hair cells of the mammalian cochlea, either the inner hair cells (IHC) or in the outer hair cells (OHC), show no evidence for electrical tuning. Unfortunately, the study of hair cells operating at frequencies much above 10 kHz is hampered by the limits of the recording bandwidth. OHCs at the high frequency cochlear base in particular are small and do not survive well once isolated, for the larger surface to volume ratio means that the cells run down, losing internal K^+ and then depolarising quickly. Consequently, much less is known quantitatively about mammalian OHC biophysics from the basal end of the cochlea and information must often be extrapolated from the more amenable low frequency cells or from more accessible but developmentally less mature cells.

Tonotopic basolateral currents: inner hair cells (IHC)

(Figure near here)

IHCs express the BK(Ca) α subunit along the length of the cochlea. The channel is expressed in plaques at the neck of the cell away from the Ca^{2+} channels at the synaptic pole [6]. This distribution impedes any electrical resonant mechanism. Despite being relatively uniform in size along the cochlea, IHCs do have measurably different gene expression profiles. Single cell transcriptomic analysis in the adult mouse suggests an increase of the Ca^{2+} buffer parvalbuminb (PARVB) in the apical cells [7]. Functionally this correlates with the observation that apical IHCs cells have faster kinetics than basal cells, even though the magnitudes of their K^+ currents are approximately the same and indeed, the resting membrane potential of both apical and basal IHCS in normal extracellular Ca^{2+} are indeed very similar (approx -70 mV) [8].

Two IHC currents noticed in early recordings can be identified. The current with the fastest kinetics, I_{Kf}, is a BK(Ca) current. I_{Kf} is sensitive to classical pharmacological blockers including TEA and iberiotoxin. It is found in all IHCs. As a definitive demonstration, deletion of the alpha subunit BK(Ca)α removes this current [9]. A second K^+ current, I_{Ks}, with slower kinetics , is also present both in cochlear cultures and in isolated adult cells. Described originally in OHCs and termed I_{Kn} [10], it is identified as the current arising from the gene KCNQ4/Kv7.4, [11]. As found in OHCs, KCNQ4 is tonotopically organised in IHCs and shows higher expression at the cochlear base [12].

Several KCNQ4 splice variants exist [13] and this may underlie the observation that the activation of the current also varies tonotopically: apical IHCs currents activate at more positive potentials [8], basal IHC at more negative potentials. The combination of more Ca^{2+} buffer and less KCNQ4 at the apex leaves IHC currents there dominated by BK(Ca); such IHCs have shorter membrane time constants and may therefore be better adapted for low frequency stimulus tracking [8]. The lower buffering capacity at the base also has in parallels IHC synaptic exocytosis for release is susceptible

to additional EGTA added during recording [14]. Such synaptic buffer effects can be understood by using reaction diffusion approaches methods to model Ca^{2+} movements at the synapse [15].

Tonotopic basolateral currents: outer hair cells (OHC)

A notable feature of the mammalian cochlea is that the OHCs, running longitudinally in three rows, shorten in cell body length (and in hair bundle length) towards the cochlear base. It might be thought that the decreased surface area might reduce input conductance, but the opposite occurs: the expression of K^+ channels per cell increases [16].

The OHC currents depend on the expression of BK(Ca)α and KCNQ4 / Kv7.4. In mice and rats, the level of BK(Ca)α expression in apical OHCs is low and progressively increases towards the cochlear base [17]. In an animal with relative low frequency hearing, such as the guinea pig or the gerbil, BK(Ca) is clearly present in apical OHCs [16]. The channel expression is associated in particular with the efferent synapse [18] and peaks mid cochlea.

As in IHCs, the dominant OHC current, I_{Kn}, derived from KCNQ4/Kv7.4, increases significantly in magnitude from apex to base of the cochlea. This is shown both by whole cell recording [16] [19] and is corroborated by immunohistochemistry[6] [20]. The current is half-activated near –80 mV and is essentially fully activated in OHCs at their resting potential. The negative activation ensures that the basal cochlear OHCs have reduced membrane time constants and the has been used as argument that OHC force generation is <u>not</u> limited by the low pass characteristics of the membrane [19]. Additional modelling studies suggest that there could even be an optimal time constant, tonotopically organised, for, if the value is too small, the phasing of the OHC force generation may be inappropriate for full enhancement of cochlear mechanics [21].

An incompletely resolved question is how the half-activation of I_{Kn} both in IHCs and OHCs, can shifted by approximately -50 mV from that measured in expression systems [22]. Explanations have included that there may be an unidentified subunit, or that the shift arises from charge effects near the membrane, for example due to PIP_2 [23]. A more recent proposal is that KCNQ4 channels cluster, the cooperative effect being to increase the open probability of the channel at rest [20].

A third K^+ current in OHCs is the small conductance K(Ca) channel SK2/KCNN2. This the sole member of the KCNN family expressed in hair cells [24]. The expression of SK2 tracks the density of innervation by the descending efferent system [17] and is maximal around the mid-basal region, tapering off towards either end. As part of the efferent system, the OHC basal pole expresses a Ca^{2+} permeable α9/α10 acetylcholine receptor: when activated, the resulting rise in intracellular Ca^{2+} can activate both SK2 and BK(Ca), hyperpolarising the cell but also providing a conductive membrane shunt [18] .

The K^+ channel BK(Ca) plays a role in overall OHC stability. Deletion of the α subunit, but not the conventional BK(Ca) β1 subunit, produces a progressive loss of high frequency OHCs but only after cochlear maturation [9]. In addition, low frequency cochlear regions become susceptible to noise damage [6]. Part of this puzzle may be that the α subunit requires an additional regulating cofactor, LRRC52 (the γ2 accessory subunit) , for stability [25], [26] and the manner in which this component interacts with the channel may allow a Ca^{2+} independent activation of the channel. Without it the cell would progressively depolarize. The multiple dependencies of long-term maintenance function of the hair cells on BK(Ca) remain to be fully elucidated.

Prestin in outer hair cells.

The so-called 'motor' protein prestin/SLC26A5 is located on the lateral membrane of OHC. Orthologs are found in all hair cells, both in vertebrate species [27] as well as in invertebrates [28], where its primary role seems to be that of a bicarbonate (HCO_3^-) transport protein acting as a pH regulator. In mammalian OHCs prestin transforms the cell into a force producing element which controls cochlear micromechanics and augmenting the frequency selectivity of the cochlea. Prestin can generate forces well into the acoustic range [29].

The density of prestin in the OHC plasma membrane, estimated from capacitance measurement and electron microscopy, shows little difference between apical and basal OHCs. In adult cells, prestin appears as a densely packed array of 10 nm diameter particles, each thought to represent a tetramer. The particle density has been estimated to be around 4000 μm^{-2} but with a large margin of error. Measurements of the charge movement, presenting a method of counting single prestin molecules, provides an estimate of ~ 10,000 μm^{-2} although this may be an underestimate [30].

The core unresolved issue is how prestin structure contributes to the generation of force in OHCs. Modelling studies suggest that SLC26A5 possesses 14 transmembrane spanning regions [31]. This receives support from high resolution, but not-atomic level, studies [32]. Although SLC26A5 tetrameric structure has not been described at an atomic resolution, the structure for the related SLC26A9 shows that this member at least is an obligate dimer, with association between the monomers forming not in the membrane but between the cytoplasmic regions [33]. It is reasonable to assume that prestin is a dimer of dimers, with the cytoplasmic STAS domain forming a critical role. The issue is contentious: molecular modelling concludes instead that a dimeric structure does not involve the STAS domain [34]. There may instead be a rigid conformational movement of transmembrane helices 3 and 10 in a manner similar the gating charge movement in ion channels [35].

Other channels in hair cells.

OHCs have both afferent and efferent nerve supplies where VGCCs are critical. The afferent synapse depends on the Ca^{2+} channel, CaV1.3, but with possible minor contributions from CaV1.4 or CaV 3.1 (see [36]). In mouse IHCs the Ca^{2+} currents increase by approximately 30% in mid-cochlea, most probably reflecting the increase in afferent synapse numbers in this region [37].

Both IHCs and OHCs express ATP activated purinergic receptors. The ionotropic P2X2/3 receptor reported on mammalian hair cells appears to be mainly localised at the apical membrane of the cell [38] and as well as postsynaptically on the Type II afferents innervating the OHCs. There are robust Ca^{2+} responses obtained in adult mouse cochleas mainly from the apical regions when ATP is applied [39] but tonotopic expression has not been reported. The metabotropic P2Y receptors are restricted to the supporting cells [40] although there is some evidence from the immunohistochemistry for basolateral hair cell expression. The RNA transcripts of several TRP channels are expressed in hair cells [41] and it has been reported that TRPV1 antagonists block K^+ currents in isolated apical OHCs [42]. There is no evidence for so far for tonotopic organisation.

Developmental factors determining tonotopy

During development, the cochlea elongates and then there is a wave of cells exiting the cell cycle, indicated by the expression of the transcription factor $p27^{KIP1}$ starting from the apex and progressing to the cochlear base. The cells subsequently differentiate to exhibit hair cell markers progressively but in the opposite direction, from base to apex. These markers include cell morphology and the

distinction between the single row of IHCs and triple rows OHCs. It also includes the emergence of the K^+ channel densities discussed above.

It has recently been shown that a Ca^{2+} signal during development is critical for establishing the gradient of K^+ channels in both IHCs and OHCs [36]. In mice with CaV1.3 deleted, spontaneous Ca^{2+} action potentials are absent in the first postnatal week and the expression of all Ca^{2+} dependent K channels is affected. Thus BK(Ca) and SK2 channel levels are reduced but the most prominent effect is to down-regulate KCNQ4. As a result, there is almost a complete absence of a definitive K^+ channel gradient. Other cofactors in this developmental process are clearly implicated, including the transmembrane protease TMPRSS3 as a upstream regulator [43],[44] but the gene regulating network(s) involved are currently unknown.

Conclusion: a two-component cochlea?

Although it has been argued that the cochlea expresses continuous protein gradients, there is some evidence for a functional discontinuity. A prominent feature of mammalian auditory nerve tuning curves is a steep high frequency roll off (>100 dB/octave) for high characteristic frequencies (CF) fibres. Such curves usually show a low frequency component (e.g. [45]). The high frequency slope makes a transition at around 5 kHz, the slope becoming shallower (see figure 1 in [45]) so that the low CF tuning curves are both less sharp (i.e. lower Q10dB) and more symmetrical. This might suggest two distinct underlying cochlear tuning mechanisms.

This idea also emerges from evolutionary arguments. It might be suggested that the small early mammals in the Jurassic were under selective pressure to develop high frequency hearing to aid localisation cues; in this view, low frequency hearing developed as a later add-on as the cochlea evolved and lengthened. The fossil record provides little soft tissue evidence, so it can also be speculated that low frequency hearing is a residuum of yet earlier, non-mammalian mechanisms. It is quite possible to model the observed range of tuning curve shapes and a recent example shows that the apparent differences in tuning curves shape can simply arise from the variations in the shape of the cochlear scalae and the spatial variation of the cochlear partition [46]. However, fast imaging of OHCs indicates that the cells only show physiologically relevant motile responses up to about 4 kHz (or to 1.5 kHz in guinea pig) [47]. Although such data is based on whole cell stimulation of isolated OHCs, it finds support from optical coherence tomography measurements in the gerbil cochlea *in vivo* [48], where the inferred displacement of the apical cochlear partition appears to be low pass filtered as well.

Nevertheless, OHCs at the cochlear base remain difficult subjects to investigate. Cochlear structure suggests that these cells work against the load of the surrounding organ of Corti which at the cochlear base is more compact. As a result, OHCs are likely to operate as isometric force generators, rather in the way that force generation works in muscle, or even in the avian cochlea where there are many rows of OHC-like cells. It is known that OHCs can generate forces up to frequencies of at least 80 kHz when acting against a constraint [29]. In this case prestin behaves like a piezoelectric component with the basolateral membrane responding to transmembrane potential. A modelling approach shows how shorter cells can be tuned to generate power specific to their tonotopic placement [49], a mechanism enhanced if the KCNQ4/Kv7.4 channels have emergent mechanosensitive properties [20]. Although a theoretical conclusion, the result shows how in the cochlea modelling and experiment interact, even though the data of necessity are be extracted from very different experimental designs.

References

1. Lewis RS, Hudspeth AJ: **Voltage- and ion-dependent conductances in solitary vertebrate hair cells**. *Nature* 1983, **304**:538–41.

2. Art JJ, Wu YC, Fettiplace R: **The calcium-activated potassium channels of turtle hair cells.** *The Journal of General Physiology* 1995, **105**:49–72.

3. Ohmori H: **Studies of ionic currents in the isolated vestibular hair cell of the chick**. *The Journal of physiology* 1984, **350**:561–81.

4. Pyott SJ, Duncan RK: **BK Channels in the Vertebrate Inner Ear**. *Int Rev Neurobiol* 2016, **128**:369–399.

5. Mann ZF, Thiede BR, Chang W, Shin J-B, May-Simera HL, Lovett M, Corwin JT, Kelley MW: **A gradient of Bmp7 specifies the tonotopic axis in the developing inner ear**. *Nat Commun* 2014, **5**:3839.

6. Engel J, Braig C, Rüttiger L, Kuhn S, Zimmermann U, Blin N, Sausbier M, Kalbacher H, Münkner S, Rohbock K, et al.: **Two classes of outer hair cells along the tonotopic axis of the cochlea**. *Neuroscience* 2006, **143**:837–849.

7. Tang F, Chen X, Jia L, Li H, Li J, Yuan W: **Differential Gene Expression Patterns Between Apical and Basal Inner Hair Cells Revealed by RNA-Seq**. *Front Mol Neurosci* 2019, **12**:332.

8. Johnson SL: **Membrane properties specialize mammalian inner hair cells for frequency or intensity encoding**. *eLife* 2015, **4**:e08177.

9. Rüttiger L, Sausbier M, Zimmermann U, Winter H, Braig C, Engel J, Knirsch M, Arntz C, Langer P, Hirt B, et al.: **Deletion of the Ca2+-activated potassium (BK) alpha-subunit but not the BKbeta1-subunit leads to progressive hearing loss**. *Proc Natl Acad Sci USA* 2004, **101**:12922–12927.

10. Housley GD, Ashmore JF: **Ionic currents of outer hair cells isolated from the guinea-pig cochlea**. *The Journal of physiology* 1992, **448**:73–98.

11. Oliver D, Knipper M, Derst C, Fakler B: **Resting potential and submembrane calcium concentration of inner hair cells in the isolated mouse cochlea are set by KCNQ-type potassium channels**. *J Neurosci* 2003, **23**:2141–2149.

12. Beisel KW, Rocha-Sanchez SM, Morris KA, Nie L, Feng F, Kachar B, Yamoah EN, Fritzsch B: **Differential expression of KCNQ4 in inner hair cells and sensory neurons is the basis of progressive high-frequency hearing loss**. *J Neurosci* 2005, **25**:9285–9293.

13. Xu T, Nie L, Zhang Y, Mo J, Feng W, Wei D, Petrov E, Calisto LE, Kachar B, Beisel KW, et al.: **Roles of Alternative Splicing in the Functional Properties of Inner Ear-specific KCNQ4 Channels**. *J Biol Chem* 2007, **282**:23899–23909.

14. Johnson SL, Olt J, Cho S, von Gersdorff H, Marcotti W: **The Coupling between Ca2+ Channels and the Exocytotic Ca2+ Sensor at Hair Cell Ribbon Synapses Varies Tonotopically along the Mature Cochlea**. *Journal of Neuroscience* 2017, **37**:2471–2484.

15. Nakamura Y, Harada H, Kamasawa N, Matsui K, Rothman JS, Shigemoto R, Silver RA, DiGregorio DA, Takahashi T: **Nanoscale distribution of presynaptic Ca(2+) channels and its impact on vesicular release during development**. *Neuron* 2015, **85**:145–158.

16. Mammano F, Ashmore JF: **Differential expression of outer hair cell potassium currents in the isolated cochlea of the guinea-pig**. *J Physiol (Lond)* 1996, **496 (Pt 3)**:639–646.

17. Wersinger E, McLean WJ, Fuchs PA, Pyott SJ: **BK channels mediate cholinergic inhibition of high frequency cochlear hair cells**. *PLoS ONE* 2010, **5**:e13836.

18. Rohmann KN, Wersinger E, Braude JP, Pyott SJ, Fuchs PA: **Activation of BK and SK channels by efferent synapses on outer hair cells in high-frequency regions of the rodent cochlea**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2015, **35**:1821–30.

19. Johnson SL, Beurg M, Marcotti W, Fettiplace R: **Prestin-driven cochlear amplification is not limited by the outer hair cell membrane time constant**. *Neuron* 2011, **70**:1143–54.

20. Perez-Flores MC, Lee JH, Park S, Zhang X-D, Sihn C-R, Ledford HA, Wang W, Kim HJ, Timofeyev V, Yarov-Yarovoy V, et al.: **Cooperativity of Kv7.4 channels confers ultrafast electromechanical sensitivity and emergent properties in cochlear outer hair cells**. *Sci Adv* 2020, **6**:eaba1104.

21. Nam J-H, Fettiplace R: **Optimal Electrical Properties of Outer Hair Cells Ensure Cochlear Amplification**. *PLoS One* 2012, **7**.

22. Kharkovets T, Hardelin J-P, Safieddine S, Schweizer M, El-Amraoui A, Petit C, Jentsch TJ: **KCNQ4, a K+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway**. *Proc Natl Acad Sci U S A* 2000, **97**:4333–4338.

23. Leitner MG, Halaszovich CR, Oliver D: **Aminoglycosides inhibit KCNQ4 channels in cochlear outer hair cells via depletion of phosphatidylinositol(4,5)bisphosphate**. *Mol Pharmacol* 2011, **79**:51–60.

24. Kong J-H, Adelman JP, Fuchs PA: **Expression of the SK2 calcium-activated potassium channel is required for cholinergic function in mouse cochlear hair cells**. *J Physiol* 2008, **586**:5471–5485.

25. Lingle CJ, Martinez-Espinosa PL, Yang-Hood A, Boero LE, Payne S, Persic D, V-Ghaffari B, Xiao M, Zhou Y, Xia X-M, et al.: **LRRC52 regulates BK channel function and localization in mouse cochlear inner hair cells**. *Proc Natl Acad Sci USA* 2019, **116**:18397–18403.

26. Lang I, Jung M, Niemeyer BA, Ruth P, Engel J: **Expression of the LRRC52 γ subunit (γ2) may provide Ca2+-independent activation of BK currents in mouse inner hair cells**. *FASEB J* 2019, **33**:11721–11734.

27. He DZ, Lovas S, Ai Y, Li Y, Beisel KW: **Prestin at year 14: progress and prospect**. *Hear Res* 2014, **311**:25–35.

28. Kavlie RG, Fritz JL, Nies F, Gopfert MC, Oliver D, Albert JT, Eberl DF: **Prestin is an anion transporter dispensable for mechanical feedback amplification in Drosophila hearing**. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2015, **201**:51–60.

29. Frank G, Hemmert W, Gummer AW: **Limiting dynamics of high-frequency electromechanical transduction of outer hair cells**. *Proc Natl Acad Sci USA* 1999, **96**:4420–4425.

30. Mahendrasingam S, Beurg M, Fettiplace R, Hackney CM: **The ultrastructural distribution of prestin in outer hair cells: a post-embedding immunogold investigation of low-frequency and high-frequency regions of the rat cochlea**. *The European journal of neuroscience* 2010, **31**:1595–605.

31. Gorbunov D, Sturlese M, Nies F, Kluge M, Bellanda M, Battistutta R, Oliver D: **Molecular architecture and the structural basis for anion interaction in prestin and SLC26 transporters**. *Nat Commun* 2014, **5**:3622.

32. Mio K, Kubo Y, Ogura T, Yamamoto T, Arisaka F, Sato C: **The motor protein prestin is a bullet-shaped molecule with inner cavities**. *J Biol Chem* 2008, **283**:1137–45.

33. Walter JD, Sawicka M, Dutzler R: **Cryo-EM structures and functional characterization of murine Slc26a9 reveal mechanism of uncoupled chloride transport**. *Elife* 2019, **8**.

34. Chang Y-N, Jaumann EA, Reichel K, Hartmann J, Oliver D, Hummer G, Joseph B, Geertsma ER: **Structural basis for functional interactions in dimers of SLC26 transporters**. *Nat Commun* 2019, **10**:2032.

35. Lenz, D, Hartmann, J, Oliver, D: **Local electrostatics control electromotile conformational transitions of Prestin/SLC26A5**. In *43rd Annual MidWinter Meeting*. . 2020:PS 996.

36. Jeng J-Y, Ceriani F, Hendry A, Johnson SL, Yen P, Simmons DD, Kros CJ, Marcotti W: **Hair cell maturation is differentially regulated along the tonotopic axis of the mammalian cochlea**. *J Physiol (Lond)* 2020, **598**:151–170.

37. Meyer AC, Frank T, Khimich D, Hoch G, Riedel D, Chapochnikov NM, Yarin YM, Harke B, Hell SW, Egner A, et al.: **Tuning of synapse number, structure and function in the cochlea**. *Nature Neuroscience* 2009, **12**:444–453.

38. Lin SCY, Thorne PR, Housley GD, Vlajkovic SM: **Purinergic Signaling and Aminoglycoside Ototoxicity: The Opposing Roles of P1 (Adenosine) and P2 (ATP) Receptors on Cochlear Hair Cell Survival**. *Front Cell Neurosci* 2019, **13**.

39. Sirko P, Gale JE, Ashmore JF: **Intercellular Ca(2+) signalling in the adult mouse cochlea**. *The Journal of physiology* 2019, **597**:303–317.

40. Gonzalez-Gonzalez S: **The Role of Purinergic P2X and P2Y Receptors in Hearing Loss**. *J Phonet and Audiol* 2018, **04**.

41. Asai Y, Holt JR, Géléoc GSG: **A quantitative analysis of the spatiotemporal pattern of transient receptor potential gene expression in the developing mouse cochlea**. *J Assoc Res Otolaryngol* 2010, **11**:27–37.

42. Wu T, Song L, Shi X, Jiang Z, Santos-Sacchi J, Nuttall AL: **Effect of capsaicin on potassium conductance and electromotility of guinea pig outer hair cell**. *Hear Res* 2011, **272**:117–124.

43. Fasquelle L, Scott HS, Lenoir M, Wang J, Rebillard G, Gaboyard S, Venteo S, Francois F, Mausset-Bonnefont AL, Antonarakis SE, et al.: **Tmprss3, a transmembrane serine protease deficient in human DFNB8/10 deafness, is critical for cochlear hair cell survival at the onset of hearing**. *The Journal of biological chemistry* 2011, **286**:17383–97.

44. Tang P-C, Alex AL, Nie J, Lee J, Roth AA, Booth KT, Koehler KR, Hashino E, Nelson RF: **Defective Tmprss3-Associated Hair Cell Degeneration in Inner Ear Organoids**. *Stem Cell Reports* 2019, **13**:147–162.

45. Temchin AN, Rich NC, Ruggero MA: **Threshold tuning curves of chinchilla auditory-nerve fibers. I. Dependence on characteristic frequency and relation to the magnitudes of cochlear vibrations**. *J Neurophysiol* 2008, **100**:2889–2898.

46. Sasmal A, Grosh K: **Unified cochlear model for low- and high-frequency mammalian hearing**. *Proc Natl Acad Sci USA* 2019, **116**:13983–13988.

47. Santos-Sacchi J, Iwasa KH, Tan W: **Outer hair cell electromotility is low-pass filtered relative to the molecular conformational changes that produce nonlinear capacitance**. *The Journal of General Physiology* 2019, doi:10.1085/jgp.201812280.

48. Vavakou A, Cooper NP, van der Heijden M: **The frequency limit of outer hair cell motility measured in vivo**. *eLife* 2019, **8**.

49. Rabbitt RD, Clifford S, Breneman KD, Farrell B, Brownell WE: **Power efficiency of outer hair cell somatic electromotility**. *PLoS computational biology* 2009, **5**:e1000444.

Figure legend.

Schematic representation of tonotopic gradients along the mammalian cochlear axis, from apex (low frequency) to base (high frequency). For comparison four decades of the frequency map (in Hz) are shown with the overlapping human (0.04-20kHz) and mouse (1-70 kHz) hearing ranges. Gradients of channel expression should be considered only as qualitative guides, with the midrange maximum in BK(Ca), SK2, and CaV1.3 reflecting cochlear innervation patterns. The tonotopic map is not absolute but should be scaled for the length of each cochlea. The basis for the gradients shown are to be found in the text.