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SYMPOSIUM

Sensory Hair Cells: An Introduction to Structure and Physiology

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Synopsis Sensory hair cells are specialized secondary sensory cells that mediate our senses of hearing, balance, linear acceleration, and angular acceleration (head rotation). In addition, hair cells in fish and amphibians mediate sensitivity to water movement through the lateral line system, and closely related electroreceptive cells mediate sensitivity to low-voltage electric fields in the aquatic environment of many fish species and several species of amphibian. Sensory hair cells share many structural and functional features across all vertebrate groups, while at the same time they are specialized for employment in a wide variety of sensory tasks. The complexity of hair cell structure is large, and the diversity of hair cell applications in sensory systems exceeds that seen for most, if not all, sensory cell types. The intent of this review is to summarize the more significant structural features and some of the more interesting and important physiological mechanisms that have been elucidated thus far. Outside vertebrates, hair cells are only known to exist in the coronal organ of tunicates. Electrical resonance, electromotility, and their exquisite mechanical sensitivity all contribute to the attractiveness of hair cells as a research subject.

Introduction

Sensory hair cells are highly specialized mechanosensitive cells found in all vertebrate animals in some related chordates (tunicates). The structure of hair cells makes them highly sensitive to displacement of the fluid environment that surrounds their apical microvilli, or stereocilia. The stereocilia are linked together and usually referred to as a hair bundle or hair cell bundle. By developing arrays of hair cells in their integument, animals can be highly sensitive to pressure waves or movement in the fluid environment surrounding the animal. In tunicates, hair cells called coronal cells are present on the velum that rings the inner surface of the oral (incurrent) siphon and may serve a protective function by sensing large particles (Caicci et al. 2007; Rigon et al. 2013). In fish, salamanders, and some anuran amphibians (e.g., *Xenopus* and other pipid frogs), a lateral line system of hair cells is sensitive to movements in the surrounding water; this is important in predator avoidance, prey detection, swimming coordination and courtship (reviewed in Ghysen and Dambly-Chaudiere 2007). The lateral line system is widely present in aquatic larval forms of frogs and salamanders; in newts it is present during the juvenile aquatic stage, disappears during the terrestrial stage, and then reappears during the adult aquatic stage (Duellman and Trueb 1994).

Terrestrial vertebrates (including land-going post-larval amphibians) lack the lateral line system but retain a highly developed vestibular system (Duellman and Trueb 1994; Hill et al. 2016). In the vestibular system, multiple sensory epithelia contribute sensitivity to seismic vibration, linear acceleration (movements generating translation in space), and angular acceleration (rotational movements of the head) (Smotherman and Narins 2004). The utricle and saccule sense linear acceleration and the semicircular canals sense angular rotation of the head (Hill et al. 2016). The amphibian saccule is also highly sensitive to seismic and auditory vibrations below about 100 Hz (Koyama et al. 1982; Smotherman and Narins 2004). Displacement of the hair bundle in the utricle and saccule results from the inertia of an overlying membrane containing a gelatinous matrix in which crystals of calcium

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carbonate are embedded (the otoconial membrane). The inertial mass of the matrix with its mineral content induces lateral bending of the stereocilia when the hair cell epithelium is displaced along the orthogonal axis. The utricular epithelium is approximately horizontal, making it most sensitive to accelerations forward, backward, or sideways. The saccular epithelium orientation is approximately parasagittal (vertical to the ground), making it most sensitive to accelerations forward, backward, upward, or downward. The otoconial membrane also induces stereociliar bending if the epithelium is displaced by tilting, and in this way hair cells of the utricle and saccule can sense postural changes of head position by the effect of gravity (Goldberg et al. 2012).

Hair cells of the semicircular canals are located in three ampullae, one for each of the canals. The tips of the hair bundles are embedded in a gelatinous cupula. Rotation of the head induces inertial pressure by the fluid within the canal against the cupula, which in turn causes displacement of the hair bundle (Goldberg et al. 2012). As an example of the sensory capability of the semicircular canal system, a housecat is able to right itself and land gracefully after being dropped from an upside-down position in less than the time it takes to fall 1.5 m. At a gravitational acceleration of 9.8 m/s^2, a cat falls 1.5 m in about 550 ms. The righting reflex which requires transduction by the vestibular receptors and rapid conversion of the transduced signal into changes of primary afferent firing frequency, followed by rapid central processing and activation of appropriate motor systems. Our understanding of the cellular mechanisms of hair cell mechanotransduction has grown rapidly in the past decade. Recent progress has been reviewed in depth by Fettiplace and Kim (2014) and Fettiplace (2017).

To summarize so far, sensory hair cells are responsible for our ability to sense angular acceleration of the head (rotational movements) by way of the semicircular canal system, linear acceleration (translational movements), and postural orientation with respect to gravity by way of the macular epithelia (in mammals, the utricle, and saccule), air-borne sound by way of the cochlea, and water displacements by the lateral line system in fish and amphibians, and water displacements by the coronal cells in tunicates. Taken together, this is an astonishing diversity of sensory functions for a single cell type, and invites a deeper exploration of the structure and function of hair cells. The remainder of this review is intended to provide an overview of the anatomy and physiology of hair cells so that a non-specialist reader may more fully appreciate the specialized reviews that follow in this collection.

Structural features of sensory hair cells

The diagnostic feature of sensory hair cells is the presence of an apical bundle of stereocilia, known as the hair bundle. Stereocilia are structurally similar to microvilli as they are packed with actin filaments in a semi-crystalline array, but stereocilia are generally larger (~200 nm diameter) than the microvilli found in other animal cells. The stereocilia number in the range of 50–100 per cell, are tightly clustered and are graded in length from one end of the bundle to the other, giving the apical membrane an axis of symmetry (Schwander et al. 2010). A single true cilium containing a $9+2$ array of microtubules is usually present just beyond the tallest stereocilia on the short-to-long axis. The kinocilium plays an important role in the development and orderly arrangement of the stereocilia, and the stereocilia closest to the kinocilium are linked to it (Fig. 1). In some hair cells, such as those in the mammalian cochlea and in the auditory papilla of some birds, there is no kinocilium in the mature hair cell. In all cases, however, a kinocilium is present during embryological development of the hair cell, and this is shown by the persistence of the ciliary basal body in the mature hair cell even in the absence of a cilium. In physiological experiments in which the kinocilium was removed from bullfrog saccular hair cells, hair cell function was not disrupted (Hudspeth and Jacobs 1979). Together these results indicate that the kinocilium is necessary for normal development of the stereocilia array but that it is not required for normal function (Schwander et al. 2010; Barr-Gillespie 2015).

The stereocilia are packed with actin filaments, as mentioned above, and the arrangement of the actin filaments is highly regular, with all of the "plus" (barbed) ends toward the stereocilium tip, which would allow the stereocilium to grow longer by addition of actin monomers at the tip. As in other cellular systems, there is turnover of actin subunits, but the rate of actin turnover is relatively low (Zhang et al, 2012). This has led to a tip-turnover model in which actin monomers at the stereocilium tip (the barbed end of the actin filaments) turn over dynamically with F-actin, while the remaining F-actin is stable and not subject to turnover (Drummond et al. 2015; Narayanan et al. 2015). Stabilization of the actin core of stereocilia involves a number of proteins believed to crosslink actin filaments and bundles of filaments together, including plastin-1,

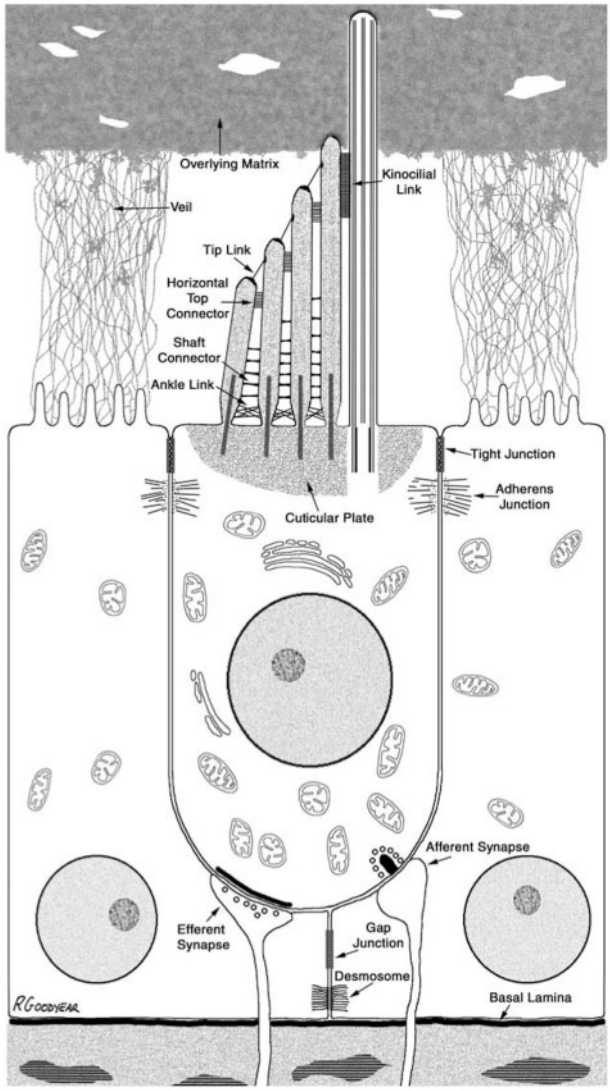

Fig. 1 Diagram of a stereotypical vertebrate hair cell, showing the major features of the cell and its relationship to the supporting cells around it. Note the presence of tight junctions linking all the cells at their apical poles and forming a tight epithelium. Note that supporting cells communicate with each other via gap junctions, but not with the hair cell. Reproduced with permission from Goodyear et al. (2006).

fascin-2, espin-3A, espin-1, and XIRP2 (Sekerkova et al. 2006; Shin et al. 2010; Taylor et al. 2015; Francis et al. 2015; Scheffer et al. 2015). Mutation or deletion of the genes for plastin-1, fascin-2, and XIRP2 result in stereocilia degeneration, confirming their importance (McGrath et al. 2017).

Importantly, the length of each stereocilium is tightly controlled to preserve the staircase-like array of stereocilia lengths, indicating that there is some sort of feedback system regulating their length (McGrath et al. 2017). Several proteins have been identified that contribute to the control of stereocilia length, including EPS8, which acts as both a bundler and a capping protein for actin filaments (Hertzog et al. 2010; Zampini et al. 2011). As expected, EPS8 is most abundant at the tips of stereocilia, as is the related protein EPS8L2 (McGrath et al. 2017). Two other proteins, myosin XVa and whirlin, are also located at stereocilia tips, and all three are required for normal stereocilia length regulation. Myosin XVa functions in part by transporting EPS8 to the stereocilia tips (Belyantseva et al. 2005; Manor et al. 2011).

At the other end, beneath the bases of the stereocilia lies a dense layer of actin filaments that form the cuticular plate, to which the stereocilia are anchored; unlike the actin in stereocilia, actin filaments in the cuticular plate appear to lack any consistent pattern of orientation. Other components of the cuticular plate include alpha-actinin, myosin Ie, myosin VI, tropomyosin, and spectrin (Goodyear et al. 2006). The protein ELMOD1 is necessary for normal development of the cuticular plate and apical membrane of hair cells; it functions by stimulating GTP hydrolysis by an ADP-ribosylation factor, ARF6 (Krey et al. 2018). Mutations in the *Elmod1* gene disrupt formation of hair bundles and lead to deafness and vestibular dysfunction in mice. The current data suggest that conversion of AFR6-GTP to ARF6-GDP stabilizes actin structures in the apical region (Krey et al. 2018).

Each stereocilium tapers to a narrow ankle region where it joins the cuticular plate, a a central group of stereocilia actin filaments extends into the cuticular plate, forming a rootlet that anchors that stereocilium (Goodyear et al. 2006). The ankle region is more flexible than the more distal regions of the stereocilium and thus forms a hinge about which the stereocilium can be deflected toward or away from the kinocilium (or toward/away from the basal body that remains where there was once a kinocilium). Lateral deflections of the hair bundle (i.e., orthogonal to the short-long axis) are also possible, at least experimentally, but such deflections do not generate a receptor potential (Fettiplace and Kim 2014).

In the extracellular space, adjacent stereocilia are linked together by a variety of connections (Goodyear et al. 2005; Fettiplace and Kim 2014). Those at the base are termed ankle links; beyond those are a series of so-called shaft connectors, and distal to the shaft connectors there are horizontal top connectors. The stereocilia adjacent to the kinocilium are linked to it by distinct kinocilial connectors. The functional effect of these multiple links is that the entire hair bundle moves as a single unit in response to mechanical stimuli. Between the tip of one stereocilium and the shaft of its adjacent, taller

neighbor there is a different mechanical link, termed a tip link (Goodyear et al. 2005). The tip links are vital to mechanotransduction and disruption of them, which can be achieved by reducing the extracellular Ca^{2+} concentration to a sub-micromolar level, results in loss of hair cell sensitivity (Assad et al. 1991). This manipulation led in turn to the hypothesis that the mechanotransducer channel is located at the tips of the stereocilia rather than at the base, a hypothesis which was eventually confirmed by high-speed, high-resolution calcium imaging (Beurg et al. 2009).

Subsequent studies have revealed the identity of many of the proteins that contribute to tip links, illustrated in Figure 2. The tip link itself is formed by cadherin 23 (CDH23) together with protocadherin 15 (PCDH15) (Siemens et al. 2004; Kazmierczak et al. 2007; Müller 2008; Alagramam et al. 2011). The upper portion of the tip link is a parallel dimer of CDH23, while the lower portion is a parallel dimer of PCDH15 (Narui and Sotomayor 2018). Multiple isoforms of PCDH15 can be created by alternative splicing and they have different spatial and developmental distributions in hair bundles. The PCDH15-CD2 isoform is functionally essential for mature tip links (Pepermans and Petit 2015). At the upper end of the tip link, CDH-23 is connected to a complex of harmonin and myosin VIIa (Fettiplace and Kim 2014). Mutations of CDH-23, harmonin, or myosin VIIa disrupt mechanotransduction (Di Palma et al. 2001; Grillet et al. 2009; Yu et al. 2017).

At the other end of the tip link, PCDH15 interacts with proteins TMIE and LHFPL5 (formerly known as TMHS). These two proteins have multiple transmembrane domains and may contribute to the functional mechanotransducer channel (Xiong et al. 2012; Zhao et al. 2014). Another protein, calcium- and integrin-binding protein 2 (CIB2), interacts with whirlin and myosin VIIa; mutations in CIB2 cause postnatal regression of stereocilia and hair cell death in the mammalian cochlea but not in the mammalian vestibular system (Michel et al. 2017).

The epithelium which contains vestibular hair cells is structurally simple and is dominated by two cell types: hair cells and supporting cells (Goodyear et al. 2006). Supporting cells span the epithelium from its apical border to the underlying basement membrane. Each hair cell is surrounded by supporting cells and is thus physically isolated from nearby hair cells. In addition, the basolateral membrane of each hair cell is separated from the basement membrane by lateral projections from the supporting cells. Supporting cells and hair cells are linked by tight junctions at the apical side of the epithelium, and by adherens junctions more basally. Supporting cells are linked to one another by gap junctions, which may provide a pathway for potassium transport as well as other cell–cell communications. In addition, supporting cells are linked by desmosomes, which contribute the necessary mechanical strength to the epithelium. Supporting cells in the organ of Corti are significantly more differentiated than in the vestibular system, and include many distinct types. The basal side of each *inner hair cell* is surrounded by processes of an *inner border cell* on the medial side and an *inner phalangeal cell* on the lateral (abneural) side. These processes form a calyx structure around both the inner hair cell body and its afferent nerve fiber, isolating it from the basilar membrane. On its lateral side, the phalangeal cell meets the medial side of an *inner pillar cell*; adjacent inner pillar cells form the medial wall of the *tunnel of Corti*. The lateral wall of the tunnel of Corti is formed, in turn, by a row of *outer pillar cells*. Beyond the outer pillar cells lie three rows of *Deiter's cells*, each of which supports an *outer hair cell* in a cup-like invagination at its upper end. Analogous to the inner hair cells, each outer hair cell is isolated from the basilar membrane on its basolateral surface by processes of Deiter's

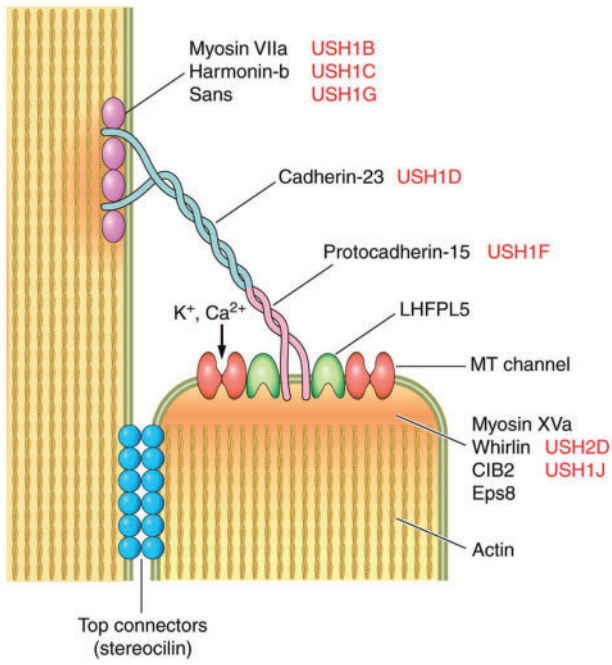

Fig. 2 Schematic depiction of two adjacent stereocilia to illustrate our current understanding of the molecules that contribute to the tip link. The "USH" designations to the right of the protein names indicate that mutations in those proteins are associated with Usher syndrome in humans. MT channel = mechanotransduction channel. Reproduced with permission from Fettiplace and Kim (2014).

cells. Lateral to the last row of Deiter's cells is a population of *Hensen's cells*, characterized by their large lipid-filled vacuoles. Unlike Deiter's cells, Hensen's cells do not make distinct contact with the basilar membrane. At the lateral extreme of the organ of Corti is a group of squamous epithelial cells called *Claudius' cells*; these cells are directly attached to the basilar membrane (Gale and Jagger 2010).

Hair cell physiology

Resting potentials

Compared to neurons in the central nervous system, the resting membrane potentials of hair cells are moderately depolarized, lying in the range of −70 to −50 mV. On the basolateral side of the epithelium, hair cells are exposed to the typical extracellular fluid of the animal; it has a high sodium concentration (120–150 mM), a low potassium concentration (2–4 mM), and a similarly low calcium concentration (2–5 mM). The primary anion in the extracellular fluid is chloride (80–110 mM). The hair cell cytosol has a low concentration of sodium (10–15 mM), a high concentration of potassium (125–140 mM), and a negligible concentration of free calcium ($\ll 1\ \mu$M) (Bosher and Warren, 1978; Sauer et al. 1999). The intracellular chloride concentration is also low (1.5–4 mM) and intracellular charge balance is due mainly to the presence of the fixed negative charges which are present on free amino acids and macromolecules (proteins and nucleic acids) at physiological pH. Assuming an intracellular K^+ concentration of 130 mM and an extracellular K^+ concentration of 3 mM, such a cell would have a resting membrane potential of about −98 mV if it behaved as a perfectly K^+-selective Nernstian membrane (where the Nernst equilibrium potential = $60*\log_{10}\{[K^+]_{out}/[K^+]_{in}\}$ in mV at 20°C). That the resting potential is so far depolarized from the K^+ equilibrium potential suggests the presence of a resting permeability to one or more additional ions which have a more depolarized equilibrium potential. Crawford and Fettiplace observed an average resting potential of about −50 mV in auditory hair cells and estimated the K^+ equilibrium potential to be −80 mV for those cells (Crawford and Fettiplace 1980). Studies of frog saccular hair cells indicate two opposing ion currents at rest, one an inward rectifier K^+ current (I_{K1}) and the other a hyperpolarization-activated inward current (I_h) (Holt and Eatock 1995). The K^+ current was activated at potentials negative to −60 mV and the inward current was activated at potentials negative to −50. Saccular hair cells fell into two morphological populations, spherical and cylindrical, which also differed in their resting potentials. Spherical cells had more depolarized potentials (ca. −50 mV) and lacked I_{K1} while possessing I_h. Cylindrical cells had more negative resting potentials (ca. −68 mV) and possessed both I_{K1} and I_h (Holt and Eatock 1995). The hyperpolarization-activated inward current I_h is a member of the HCN channel family and a recent report suggests that this type of channel is necessary for normal vestibular function (Horwitz et al. 2011). In mouse cochlear hair cells, which have a resting potential around −72 mV, voltage-sensitive K^+ channels of KCNQ type contribute strongly to the resting potential (Oliver et al. 2003). Vestibular neurons express a variety of voltage-gated, inward rectifier, and Ca^{2+}-activated channels, which contribute to the functional specialization of type I and type II hair cells (Meredith and Rennie 2016).

On the apical side of the hair cell membrane, the ionic composition of extracellular fluid in the inner ear more closely resembles the cytosol (Bosher and Warren 1978; Sauer et al. 1999). This fluid, called endolymph, is actively secreted by the stria vascularis of the scala media of the mammalian cochlea. The scala media is a fluid-filled duct which is continuous with the semicircular canals and with the fluid space that overlies hair cells in the other macular epithelia, such as the mammalian saccule and utricle. In those regions, an epithelium similar to the stria vascularis secretes endolymph (Wilms et al. 2016). The K^+ concentration of cochlear endolymph in mammals is about 150 mM and the concentration of Na^+ is about 1 mM; the Ca^{2+} concentration is about 30 μM, which is about 1% of the normal extracellular value but at the same time much higher than the normal intracellular value, which is less than 1 μM (Fettiplace 2017). Because the K^+ concentrations are similar on both sides of the apical membrane, the Nernst potential for K^+ across that membrane is close to zero ($E_K = 60*\log_{10}\{[K^+]_{out}/[K^+]_{in}\}$. Consequently, if the resting membrane potential is −50 mV (due to resting permeabilities on the basolateral side), there exists an inward driving force for K^+ to enter the cell on the apical side. Because of the low Na^+ concentration in endolymph, the driving force for Na^+ is actually outward across the apical membrane ($E_{Na} = 60*\log_{10}(1/10)$=-60 mV), while the driving force for Ca^{2+} remains inward [$E_{Ca}=(60/2)*\log_{10}(30\ \mu M/1\ \mu M) = +44$ mV]. In mammals and in some birds, there is a significant positive electrical potential between endolymph and the basolateral extracellular fluid, as a result of the active ion transport that takes place in the endolymph-secreting epithelium. In mammals, this

potential is about +80 mV in the cochlea and therefore adds to the inward driving force for cations across the hair cell apical membrane (Zdebik et al. 2009; Wilms et al. 2016). By adding to the driving force for the inward flow of cationic current, this is thought to enhance the response of hair cells to high-frequency tones (Nin et al. 2008, 2016; Fettiplace 2017).

Mechanotransduction

The appropriate mechanical stimulus for a hair cell is one that displaces the stereocilia bundle in the direction of the tallest stereocilia. This was demonstrated by Hudspeth and Corey using an isolated bullfrog saccular epithelium preparation (Hudspeth and Corey 1977). Minute displacements of the hair bundle produced depolarizing receptor potentials of several millivolts, and displacement in the opposite direction caused a hyperpolarization, though it was smaller in amplitude. Similar experiments on mammalian outer hair cells indicate that a 250 nm deflection of the hair bundle generates a half-maximal activation of the inward current and a 500 nm deflection is saturating (Fettiplace and Kim 2014). The hyperpolarizing response observed in bullfrog saccular hair cells indicates that some of the mechanosensitive channels must be open at rest, but they can only a small fraction of the total number since the cells were much more responsive in the depolarizing direction (Hudspeth and Corey 1977).

The ionic current activated by hair bundle displacement is carried by cations, and the channel is permeable to Na^+, K^+, and Ca^{2+} (Corey and Hudspeth 1979). Potasssium ions carry most of the current, because they are the most abundant cation in the endolymph; however, Ca^{2+} may contribute up to 10% of the inward current despite its low concentration (20–30 μM in cochlear endolymph, 200–250 μM in vestibular endolymph) (Ricci and Fettiplace 1998). As noted above, current carried by Na^+ will be in the outward direction; this would not contribute to the depolarizing receptor potential but might assist in Na^+ homeostasis by allowing outward diffusion of Na^+ that diffused inward on the basolateral side. Depolarization caused by inward current across the apical membrane increases the already-high conductance to K^+ in the basolateral membrane by opening voltage-gated K^+ channels; these appear to include KCNQ4 channels, and mutations of the KCNQ4 gene cause a dominant form of hereditary deafness (Kharkovets et al. 2000).

The large outward driving force for K^+ on the basolateral side of the hair cell means that no energy expenditure is required to restore the K^+ ion concentration gradient during and after the mechanotransduction process. This is a remarkable efficiency of cellular energetics and a prime example of the physiological power of epithelia. By a combination of K^+ diffusion through the perilymph and cell-to-cell diffusion through gap junctions connecting supporting cells to each other and to other cells, the K^+ that entered through the apical membrane eventually travels back to the stria vascularis to be recycled into more endolymph (Zdebik et al. 2009; Fettiplace 2017).

Synaptic transmission

Mature hair cells do not produce action potentials, so all synaptic transmission is based on graded receptor potentials. Hair bundle displacement produces inward currents as large as 10 pA for 1 nm of displacement, which would lead to a depolarization of 1 mV for a hair cell having an input resistance of 100 MΩ (Fettiplace and Ricci 2006). The physiological range for receptor potentials is from the resting potential to about −20 mV (Glowatzki et al. 2008), corresponding to hair bundle displacements of up to 50 nm under physiological conditions.

Neurotransmitter release at the afferent synapse is Ca^{2+}-dependent, as at other neuronal synapses, but there are several important specializations in the hair cell synapse. Structurally, the presynaptic zone contains a prominent, oblong, electron-dense structure around which synaptic vesicles are clustered; this leads it to be termed a ribbon synapse (Fig. 1), and the appearance in transmission electron micrographs is similar to that seen in retinal photoreceptors and bipolar cells (Wichmann and Moser 2015). The release of neurotransmitter is graded in relation to membrane depolarization and the relationship of transmitter release to Ca^{2+} influx is linear (Glowatzki et al. 2008; Rutherford and Pangrsic 2012; Fettiplace 2017). This is rather surprising, because the Ca^{2+}-dependence of vesicular exocytosis at the ribbon synapse, when measured by photolysis of photo-sensitive Ca^{2+}-buffers, seems to display the same 4th or 5th power relationship as that seen at other neuronal synapses (Glowatzki et al. 2008; Johnson et al. 2017; Rutherford and Pangrsic 2012). However it occurs, the net effect of the observed linear relationship at the ribbon synapse is to make transmitter release more sensitive to small depolarizations, thereby enhancing the overall sensitivity of the system.

In the absence of stimulus-induced hair cell depolarization, neurotransmitter is continually released due to the tonic open state of L-type

voltage-dependent Ca^{2+} channels at the active zone of the presynaptic membrane at the normal resting potential (Cho and von Gersdorff 2012). The neurotransmitter released is glutamate, and the postsynaptic receptors are fast, excitatory AMPA receptors (Sadeghi et al. 2014; Kirk et al. 2017). The result of the tonic release of glutamate is that the primary afferent fibers are firing spontaneously in the absence of hair cell activation and increase their firing rate in proportion to the graded increase of glutamate caused by graded hair cell depolarization.

The hair cell ribbon synapse is specialized to release neurotransmitter with minimal delay; this is important because many processes, such as sound source localization, require precise assessment of the interaural latency of sound arrival. How this is accomplished is not completely understood, but several key components of the ribbon synapse have been identified. Prominent among these is the protein ribeye, which forms the bulk of the ribbon structure (Wichmann and Moser 2015). Ribeye is anchored to the active zone by the protein bassoon (Fig. 3), and mice with bassoon mutations have impaired ribbon synapse function (Wichmann and Moser 2015). The identity of the proteins that tether synaptic vesicles to ribeye is not known, but may involve the B domain of ribeye (Wichmann and Moser 2015). Knockout of ribeye has recently been reported in mice, with the result that temporal precision of sound encoding was disrupted, though synaptic transmission continued (Jean et al. 2018).

Besides the structural differences from central synapses, there are significant functional differences in ribbon synapses: vesicle priming factors such as Munc13 are not involved, SNARE proteins do not appear to be necessary, and synaptotagmin, the Ca^{+2}-binding protein that triggers exocytosis at central synapses, is completely absent from hair cell ribbon synapses (Rutherford and Pangrsic 2012; Pangrsic et al. 2012). In place of synaptotagmin, ribbon synapses contain a related protein, otoferlin, which, like synaptotagmin, has multiple C2 domains which could bind Ca^{2+} and promote vesicle exocytosis (Cho and von Gersdorff 2012; Fettiplace, 2017; Michalski et al. 2017). Otoferlin is necessary for exocytosis from hair cell ribbon synapses, as knockout of the protein in mice causes deafness, and mutation of the otoferlin gene is associated with human deafness (Roux et al. 2006). Further evidence in support of otoferlin as a Ca^{2+} sensor and promoter of vesicle fusion comes from experiments using a knock-in mouse with a modified otoferlin having lower Ca^{2+} affinity in its C2 domains (Michalski et al. 2017). These mice had normal ribbon synapse structure and presynaptic Ca^{2+} currents, but synaptic exocytosis was delayed and brainstem auditory responses were smaller (Michalski et al. 2017). Otoferlin may also contribute to recruitment of vesicles to the ribbon synapse (Michalski et al. 2017). Many questions remain regarding the hair cell ribbon synapse, and this system will certainly be a focus of productive research activity for many years to come.

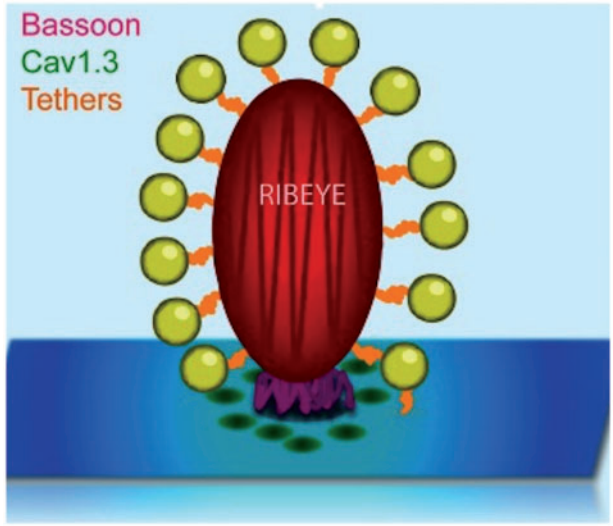

Fig. 3 Schematic depiction of the ribbon synapse in a mature inner hair cell. Synaptic vesicles (small spheres) are clustered around the ribeye protein (large oval) and are linked to it by as-yet undescribed tethers. The bassoon protein (pedestal below ribeye) is positioned between ribeye and the presynaptic membrane and may serve as an anchor for ribeye. Voltage-sensitive Cav1.3 Ca^{2+} channels (dark spots near bassoon) are clustered tightly around the ribeye-bassoon complex, minimizing the diffusion time between Ca^{2+} entry and Ca^{2+}-triggered exocytosis. Modified with permission from Wichmann and Moser (2015).

Another specialized synapse is found in Type I vestibular hair cells, which are surrounded on the basolateral side by a calyx-like expansion of the primary afferent nerve ending (Fig. 4). Both type I and type II hair cells form ribbon synapses onto the primary afferent, and it is notable that one primary afferent may form synapses with both type I and type II hair cells (Eatock and Lysakowski 2006). However, the presence of a calyx constrains the outward diffusion of K^+ from the basolateral membrane of the type I cell, leading to accumulation of K^+ in the synaptic cleft. While this seems disadvantageous to the hair cell, which must eliminate on the basolateral side the K^+ that enters through the stereocilia, it also has the potential to directly depolarize the primary afferent membrane by the resulting charge accumulation and/or by Nernstian depolarization, producing thereby a direct, non-quantal excitation

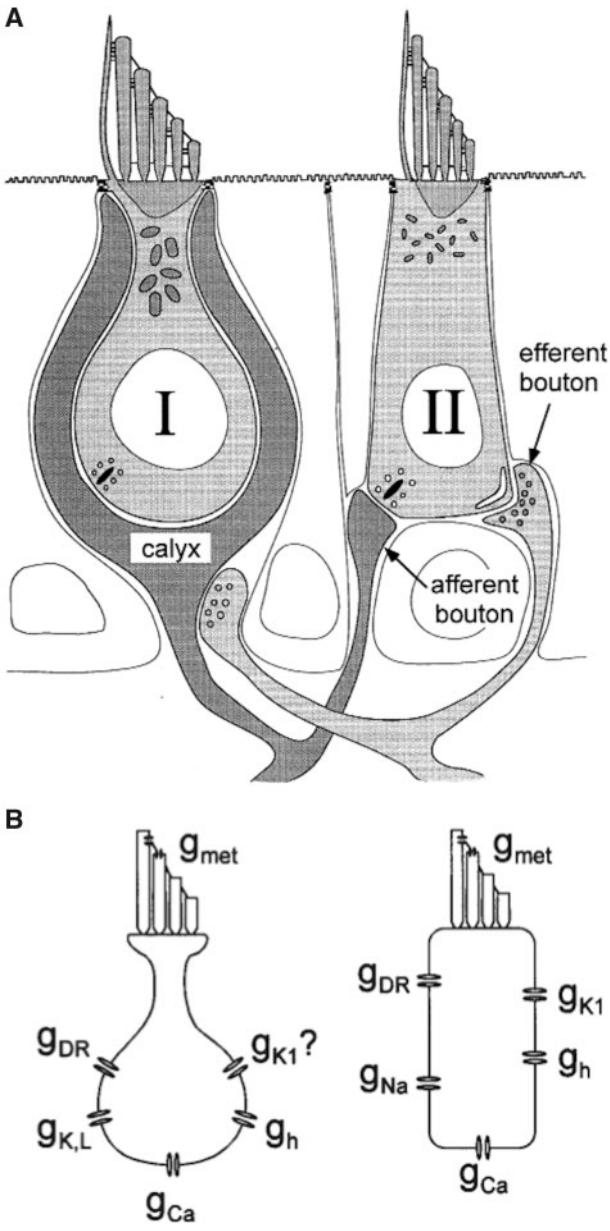

Fig. 4 Diagram of stereotypical type I and type II hair cells from a mammalian vestibular organ. (**A**) Note that a single primary afferent may synapse with both a type I and a type II hair cell. A single efferent neuron may synapse onto a type I afferent ending (a postsynaptic synapse) and may synapse onto a type II hair cell (a presynaptic synapse) or onto the afferent axon (a postsynaptic synapse, not illustrated). But an efferent neuron cannot synapse onto a type I hair cell directly. (**B**) Ionic conductances that have been identified in vestibular hair cells. GDR, delayed rectifier K^+ conductance; GK, L, K^+ leak conductance; GCa, voltage-sensitive, noninactivating Ca^{2+} conductance; GNa, TTX-sensitive, voltage-sensitive Na^+ conductance; GKI, K^+- selective inward rectifier; Gh, hyperpolarization-activated inward current; GA, voltage-sensitive, rapidly inactivating K^+ conductance, or A-current; GDRI, delayed rectifier K^+ conductance; GDRII, delayed rectifier K^+ conductance; GMET, mechanoelectrical transduction conductance. Reproduced with permission from Eatock et al. (1998).

(Songer and Eatock 2013). Consistent with this idea, the calyceal afferent membrane contains voltage-sensitive KCNQ-type channels, providing both a Nernstian permeability pathway and an exit channel from the synaptic cleft for accumulated K^+ (Songer and Eatock 2013).

The mechanotransducer channel

Although the mechanotransducer channel has not yet been fully isolated and characterized, much is known about it. Early experiments used low extracellular Ca^{2+} (less than 1 μM) to cause partial separation of the stereocilia tip links. Under fortunate conditions, this leaves enough intact tip links to allow electrical recording from one or a few channels at a time, and thus measurement of the channel conductance. When the extracellular Ca^{2+} concentration was subsequently raised to about 3 mM, the channel conductance measured was on the order of 100 picosiemens (100 pS) (Ohmori 1985; Crawford et al. 1991; Beurg et al. 2006). In contrast, most voltage-gated ion channels have conductances in the range of 10–30 pS (Hille 2001). Even more surprising, the conductance of the mechano-sensitive channel nearly doubled when the extracellular Ca^{2+} was lowered to its normal (sub-millimolar) endolymph concentration (Crawford et al. 1991). The increased conductance in low Ca^{2+} suggests that Ca^{2+} has an inhibitory effect on the channel (Beurg et al. 2010).

The mechanosensitive ion channel was conclusively localized to the tips of stereocilia by calcium imaging with fluorescent dyes (Lumpkin and Hudspeth 1995; Beurg et al. 2009). This result is consistent with the presence of tip links between adjacent stereocilia, which provide a possible mechanism for the activation of tip-located mechanoreceptors: they could act as a gating spring. Several components of the tip links have been identified, and among them two essential proteins are cadherin23 (CDH23) and protocadherin 15 (PCDH15) (Siemens et al. 2004; Narui and Sotomayor 2018). Both CDH23 and PCDH15 are calcium-binding proteins that form calcium-dependent links, so their presence is consistent with the earlier observation that low extracellular Ca^{2+} disrupts tip links and blocks mechanotransduction (Assad et al. 1991).

It is now well-established that CDH23 forms the upper region of the tip link and PCDH15 forms the lower (Zhao and Müller 2015). Subsequent searches, based on genes responsible for a severe deafness syndrome (Usher Syndrome Type 1, or USH1), have led to the description of proteins that engage with

CDH23 or PCDH15 and could possibly form the mechanotransducer channel. These searches were productive and yielded important components of the stereocilia tip-link complex, including harmonin, sans, and myosin VIIa, which form a complex at the upper end of the tip-link and interact with CDH23. Also identified were CIB2, TMIE, and LHFPL5 (also known as TMHS), which form a complex at the lower end of the tip link and interact with PCDH15 (Zhao and Müller 2015). Both TMIE and LHFPL5 are integral membrane proteins and could possibly contribute to a transmembrane ion channel. However, experiments with a mouse knock-out of LHFPL5, while showing reduction of the mechanotransduction conductance, indicated not that LHFPL5 was a component of the mechanotransducer channel, but rather that disruption of LHFPL5 acts by downregulating expression of another potential channel protein, TMC1 (Beurg et al. 2015).

Multiple lines of evidence suggest that TMC1 is part of the mechanotransducer channel (Holt et al. 2014). Like TMIE and LHFPL5, TMC1 is localized to the stereocilia tips (Kurima et al. 2015). Also, TMC1 interacts with CIB2, which itself is necessary for normal mechanotransduction (Giese et al. 2017). Experiments with mice expressing TMC1, the related protein TMC2, or a mutated TMC1 showed that the votage-clamp recording from inner hair cells or vestibular hair cells yielded normal-appearing currents with either TMC1, TMC2, or a mixture of the two, while the mice expressing mutated TMC1 only showed reduced currents (Pan et al. 2013). These data do not prove that TMC1 or TMC2 can form a complete channel, but they strongly suggest that TMC proteins contribute to the channel pore (Pan and Holt 2015). However, this does not necessarily imply that they are the only components of the channel pore. Many questions remain and the issue is unlikely to be fully resolved until a functional mechanotransducer channel can be reconstituted in a heterologous expression system (Fettiplace 2016; Corey and Holt 2016; Wu and Müller 2016).

Reverse-polarity transduction

In hair cells lacking both TMC1 and TMC2, or in hair cells in which the tip links have been thoroughly disrupted, it is possible to observe a reverse-polarity current when the hair bundle is displaced in the negative direction (away from the tallest stereocilia). This outward current results from deformation of the apical plasma membrane of the cell (Beurg et al. 2016). Subsequent investigation determined that the mechanosensitive channel responsible is piezo2, which has previously been shown to underlie mechanotransduction in mammalian touch sensation (Ranade et al. 2014; Beurg and Fettiplace 2017). However, the normal role of this channel in the apical membrane of hair cells is not known.

Adaptation

All sensory systems exhibit adaptation, which is a decline in sensory response to a steady or unchanging stimulus. Adaptation may occur at any level of the system, from peripheral receptor to central integrating neural circuit, and it often occurs at multiple levels. From the organism's point of view, adaptation allows the sensory system to remain sensitive to *changes* or *differences* in the environment, as those are the locus of information that is useful for survival and reproduction. Like many other mechanosensory cells, hair cells show rapid adaptation to tonic displacement of the stereocilia bundle. This is functionally important in the vestibular system because it allows hair cells to sense changes of acceleration against a background of constant velocity. Additionally, in auditory hair cells of amphibians, reptiles, and birds, adaptation contributes to frequency sensitivity. Two general types of hair cell adaptation have been described: fast and slow. Both were originally described in amphibians (frog saccule) or reptiles (turtle auditory papilla), and both types depend on Ca^{2+} influx through the mechanosensitive channel (Assad et al. 1989; Wu et al. 1999; Eatock 2000; Colclasure and Holt 2003; Corns et al. 2014)

Fast adaptation occurs very rapidly, with a time constant of <2 ms, and it includes a rapic, active movement of the stereocilia against the direction of the stimulus (i.e., toward the shorter stereocilia) (Crawford et al. 1989). The recoil has the same time course as the decay of inward current following stereocilia displacement, and the most likely mechanism is that entering Ca^{2+} binds to the mechanotransducer channel complex and causes rapid channel closing (Crawford et al. 1989; Ricci et al. 2000a; Fettiplace and Ricci 2003; Stepanyan and Frolenkov 2009). Consistent with this idea, turtle auditory hair cells contain a high concentration of endogenous Ca^{+2} buffer, equivalent to 0.1–0.4 mM BAPTA, and the concentration of endogenous buffer is higher in high-frequency responding hair cells than in low-frequency cells. In parallel, the time constant of fast adaptation is longer in high-frequency cells than in low-frequency cells (Ricci et al. 1998).

Fast adaptation has also been observed in outer hair cells of the mammalian cochlea. The kinetics

of fast adaptation were more rapid than in turtle hair cells, even when both were at room temperature, with the average time constant for fast adaptation in mouse cells around 154 μs when the extracellular Ca^{2+} concentration was 1.5 mM. When extracellular Ca^2 was reduced to 50 μM, a physiological concentration, the time constant was slower (620 μs) and the total inward current was greater (Kennedy et al. 2003). These differences reflect the fact that the mechanotransducer channel is blocked by millimolar Ca^{2+}, which was also observed in turtle hair cells (Crawford et al. 1991). Similar results were obtained by Corns and colleagues when using millimolar Ca^{2+}, but when Ca^{2+} was lowered to a physiological concentration, both fast and slow adaptation were abolished (Corns et al. 2014). It has also been reported that fast adaptation can occur independent of Ca^{2+} entry in mammalian cochlear hair cells (Peng et al. 2013).

Slow adaptation occurs with a longer time constant (5–50 ms). This adaptation involves a passive adjustment of the hair bundle in the direction of the excitatory displacement (i.e., toward the longer stereocilia). In the process, the channels become reset to respond to further displacement, and this is thought to involve adjustment of the resting tension on the channels. One model for this is that Ca^{2+} binds to myosin and causes its detachment from an actin filament in the stereocilium. The myosin is hypothesized to be attached to an elastic element, or "gating spring," which is also attached to the channel, so that detachment of the myosin from actin would allow the complex to slip in the direction that reduces tension on the gating spring. Following channel closure, intracellular Ca^{2+} returns to normal and the myosin could again bind to actin and migrate in the opposite (tension-increasing) direction. Once tension reaches the point for channel opening, Ca^2 influx would again act to reduce the tension. In this way, a negative feedback loop would keep the channels optimally positioned for sensitivity (Hudspeth et al. 2000; Farris et al. 2006). Recent evidence in support of this model is that directed mutation of the myosin1c gene, combined with an ADP analog that interferes with the mutated myosin, selectively blocks slow adaptation in the transgenic mice carrying the mutation (Holt et al. 2002). It has also been reported that myosin VIIa is necessary for slow adaptation in mouse cochlear hair cells (Kros et al. 2002). Myosin VI has also been implicated in adaptation (Marcotti et al. 2016).

How do auditory hair cells distinguish tones?

Through hair cells, the auditory system encodes sound intensity (loudness) and sound frequency (tone). In contrast to the lateral line and vestibular systems, in which water or endolymph translates in the plane of the epithelium, auditory transduction involves vertical displacements of the hair cell (i.e., along the basolateral-to-apical axis) caused by sound-induced oscillation of the epithelium. It is straightforward to see how larger amplitudes of oscillation might be induced by louder sounds, and how this could create greater depolarization of the hair cell. But how does the ear distinguish tones? Two fundamentally different mechanisms have evolved for tone discrimination; one which employs electrical resonance in the basolateral membrane of the hair cell, while the other makes use of tonotopic differences in the mechanical resonance of the basilar membrane. There are also tonotopic differences in the stiffness of hair bundles.

In amphibians, reptiles, and birds, tone discrimination derives primarily from resonant electrical properties of the hair cells; some resonate at lower frequencies and some at higher frequencies (Art et al. 1995; Art and Fettiplace 1987; Fuchs et al. 1988; Smotherman and Narins 1999a, 1999b). The physiological mechanism for this resonance involves an intriguing interplay of voltage-activated Ca^{2+} channels and Ca^{2+}-activated K^+ channels. This was initially described by Fettiplace and colleagues (Crawford and Fettiplace 1981; Art and Fettiplace 1987, 2006; Goodman and Art 1996). The electrical resonance manifests as a damped oscillation of membrane potential in responses to an applied current pulse, or a sustained oscillation of membrane potential in response to pure tone stimulation of the intact cochlea. The resonant response to pure tones is not an all-or-none phenomenon; each cell displays a tuning curve with maximal resonance at a characteristic frequency and a graded decline with frequencies on either side (Art et al. 1985). What is remarkable is that the same characteristic frequency can be seen in the isolated hair cell: it is an intrinsic property of the cell membrane.

While differences in the density and kinetics of channels bearing inward or outward current could influence a resonant feedback loop, the experimental evidence indicates that most of the differences lie in the density and kinetics of BK potassium channels, which are sensitive to both voltage and Ca^{2+} (Art and Fettiplace 1987; Art et al. 1995). Cells with a lower resonant frequency have a lower density of BK channels and those channels are composed of subunits that result in slower activation of the channel. Thus, the membrane response to both depolarization and Ca^{2+} influx are slowed, resulting in a lower resonant frequency. Cells which have a higher density of BK channels, and in which the channel subunits yield faster kinetics, repolarize more rapidly

after an initial depolarization and Ca^{2+} influx. Buffering of the cytosolic Ca^{2+} then allows the BK channels to close, causing a rebound depolarization and Ca^{2+} channel opening. In the presence of tones that match the resonant frequency range of the cell, this would yield enhanced receptor potentials compared to a cell lacking resonance (Wu et al. 1995).

In addition to membrane electrical resonance of individual cells, there is an overall tonotopic organization in the basilar (auditory) papilla of reptiles such as turtles (from which many of these studies are drawn). Hair cells with high-frequency resonance reside at the basal end and cells with progressively lower-frequency resonance lie toward the apical end of the epithelium. This is similar to the the organization of the mammalian cochlea, and raises the question of whether the basilar papilla membrane itself has mechanical resonance properties like the basilar membrane in the mammalian organ of Corti (Ricci et al. 2000). If that were the case, the electrical resonance of the hair cells could be viewed as increasing the ear's existing sensitivity to specific tones, effectively acting as a gain amplifier. The question was explored in the turtle ear by laser interferometry, the result that the turtle basilar membrane appears to be broadly tuned and does not display intrinsic tonotopic resonance differences (O'Neill and Bearden 1995). Similar results have been reported from the chick cochlea (Xia et al. 2016). These results are consistent with earlier experiments in alligator lizards which used the Mossbauer source method (Peake and Ling 1980).

While the basilar membrane is not tonotopically tuned in non-mammalian species, there are differences in the morphology of stereocilia along the length of the basilar papilla, one of which is stereocilia length (Howard and Ashmore 1986; Fettiplace and Fuchs 1999; Fettiplace 2017). These differences affect the passive compliance of the stereocilia, to which the opening and closing of the mechanosensitivie channels contribute an active component (i.e., greater compliance when the channels open and reduced compliance when the channels close). The features contribute mechanical tuning to hair cells that adds to their electrical tuning (Fettiplace and Ricci 2006).

Besides enhancing frequency discrimination, membrane electrical resonance can also amplify hair cell responses by inducing oscillatory movements of the hair bundle (Martin and Hudspeth 1999; Ricci et al. 2000). Consistent with this, short hair cells of the bird auditory papilla display electrical tuning although they have no afferent function (Tan et al. 2013). The time constant of fast adaptation is related to the resonant frequency, and the active movement of the hair bundle caused by fast adaptation could generate enough force to enhance displacement of hair bundles in long hair cells (the bird analog of inner hair cells) (LeMasurier and Gillespie 2005; Tan et al. 2013).

It is likely that electrical resonance first evolved in vestibular hair cells and was further refined in the tetrapod evolution of auditory papillae. In that light it is worth noting that electrical resonance properties are also found in the vestibular neurons of mammals, where they contribute importantly to rapid perception of head movements (rotational or translational) at physiological frequencies (Vollrath and Eatock 2003; Eatock and Lysakowski 2006; Fisher et al. 2011; Songer and Eatock 2013; Venturino et al. 2015).

Auditory hair cells also have structural features that vary tonotopically along the length of the basilar membrane in the mammalian cochlea. Chief among these is the length of the stereocilia. Hair cells toward the apex, which are most sensitive to low frequencies, have longer stereocilia, and hair cells toward the basal end, sensitive to high frequencies, have shorter stereocilia. In rat cochlea, inner hair cells toward to apex with an expected best frequency of 4 kHz had stereocilia that were twice as long as those in hair cells with an expected best frequency of 30 kHz (Furness et al. 2008). These differences are likely to enhance frequency sensitivity, as the stiffness of stereocilia is inversely proportional to the square of their length in frog sacculus and turtle cochlear hair cells (Crawford and Fettiplace 1985; Howard and Ashmore 1986).

In contrast to vestibular hair cells and non-mammalian auditory hair cells, cochlear hair cells of mammals do not display electrical resonance. In the absence of electrical resonance, mammalian inner ears have evolved two complementary mechanical features to enhance tone discrimination and sound sensitivity. The first is that the basilar membrane changes in stiffness along its length, being more stiff toward the basal end and less stiff toward the apical end. This arises from differences in the width of the basilar membrane and also differences in its structure, which is thicker at the base and thinner toward the apex. As initially observed by von Bekesy on the inner ears of human cadavers, sound entering the cochlea generates traveling waves along the length of the basilar membrane, and the amplitude of displacement is tonotopically organized; the apical end resonates with lower frequencies and the basal end at higher frequencies (von Bekesy 1960). Traveling waves of this sort have not been observed in fish, amphibians, reptiles, or birds.

The second mammalian innovation is the introduction of a second type of hair cell to amplify the basilar membrane's oscillation at specific sound frequencies. These are the outer hair cells. The cochleas of eutherian mammals comprise one row of primary sensory hair cells (inner hair cells, IHCs) and three rows of modulatory hair cells with little or no afferent function (outer hair cells, OHCs). Each inner hair cell receives afferent synapses from 10 to 15 primary afferent nerve fibers, which amounts to 90–95% of the primary afferent fibers. The outer hair cells share synaptic connection to the remaining 5–10% of afferent axons. The upshot is that the CNS auditory system oversamples the activity of IHCs by a many-to-one mapping, while averaging the activity of OHCs by a one-to-many mapping. This arrangement indicates that IHCs are the primary mediators of auditory information.

Structurally, the OHCs have more stereocilia and the W-shaped arrangement of the stereocilia is more pronounced (Furness and Hackney 2006). In addition, the tips of OHC stereocilia are physically embedded in the overlying tectorial membrane, while those of IHCs are not. The functional significance of this arrangement became apparent when William Brownell and colleagues discovered that OHCs could shorten along their base-to-apical axis when depolarized (Brownell et al. 1985). This shortening is extremely rapid and can be observed in response to imposed depolarizations at frequencies up to 100 kHz, near the top end of frequency sensitivity in bats and cetaceans (whales and dolphins). Impressively, the response amplitude and response phase of OHCs display high fidelity out to 50 kHz in vitro (Frank et al. 1999). By shortening, the OHCs increase the shearing motion of the tectorial membrane over the surface of the IHCs, thereby amplifying the displacement of the IHC stereocilia bundle.

Shortening of the OHC is accompanied by proportional increase of cell radius; the cell volume remains constant. Uniquely among vertebrate cells, OHCs have a positive turgor pressure; this is made possible by structural specializations of the cuticular plate at the cell's apical pole and a highly regular, thick cytoskeletal layer lining the lateral and basolateral plasma membrane (Brownell 2006). Shortening of the OHC under natural conditions begins with displacement of the stereocilia bundle and opening of mechanosensitive channels at their tips. Depolarization-induced shortening results from activation of a piezoelectric effect, meaning that electrical depolarization and physical shortening are directly coupled. The protein responsible for this effect has been cloned and identified and named prestin (Dallos and Fakler 2002; Dallos et al. 2006; Dallos 2008). Prestin belongs to a family of sulfate transporters called Slc26A (Vincourt et al. 2003). Like its family members, prestin has a large number of putative transmembrane domains predicted to form alpha helices with mostly hydrophobic amino acid residues. But unlike its cousins, prestin preferentially binds Cl^-, not SO_4^{2-}, and it does not function as a transporter. Our current knowledge of prestin structure and function has been recently reviewed (He et al. 2014). Intracellular Cl^- is required for normal OHC function, consistent with a model in which Cl^- ions bind to prestin and act as the voltage sensor. Chloride ions would be predicted to move away from the cell interior during hyperpolarization and toward the cell interior during depolarization. The close coupling between charge movement and cell shortening (actuation) remains unresolved. Prestin has also been reported to contribute to amplification by short hair cells in the bird auditory papilla (Beurg et al. 2013).

One constraint of the prestin-based cochlear amplifier is that the ability of an OHC to keep pace with high frequency tones is limited by the time constant of the OHC membrane, because OHC shortening must be preceded by OHC depolarization. Since the membrane time constant is the product or membrane resistance and membrane capacitance ($\tau = R_m \times C_m$), one approach to solve this problem is to reduce R_m. This is accomplished in OHCs by increasing the concentration of endogenous intracellular Ca^{2+} so that about 50% of mechanotransducer channels are open at rest, which in turn depolarizes the OHC membrane potential to about −40 mV (Johnson et al, 2011). An additional effect reducing R_m is that, at −40 mV, voltage-dependent KCNQ4 channels on the OHC basolateral membrane are fully activated. This creates a "silent current" of K^+ through the OHC, which may be energetically expensive but which yields high sensitivity across the frequency spectrum (Johnson et al. 2011; Nam and Fettiplace 2012; Fettiplace 2017).

Efferent modulation of the cochlear amplifier

Hair cells generally receive both afferent and efferent innervation. In mammals, the OHCs receive about 90% of the efferent innervation. Whether OHCs even contribute an afferent signal is an open question, as attempts to record afferent activity in response to OHC activation have yielded negative results. In bird cochlea, the short hair cells, which appear analogous to OHCs, receive only efferent

innervation. So, what is the function of efferent synapses on hair cells?

The transmitter released at the efferent synapse has long been known to be acetylcholine (ACh), and the postsynaptic receptor for ACh in hair cells is of the nicotinic type, which has a ligand-gated channel. In most cells, such as skeletal muscle, the ACh-gated channel is a non-selective cation channel, permeable to Na^+, K^+, and Ca^{2+}. The efferent synapses are on the basolateral membrane of hair cells, where the predominant extracellular cations are Na^+ and Ca^{2+}, both of which would produce inward, depolarizing currents (Glowatzki and Fuchs 2000; Oliver et al. 2000). Yet, the effect of efferent signaling is a hyperpolarizing, inhibitory synaptic potential (Brown and Nuttall 1984). How does this come about?

Like other nicotinic receptors, the receptor in hair cells is a pentamer. It has five subunits but they need not be identical. Two nicotinic subunit types, alpha-9 and alpha-10, have been identified in hair cells, and when expressed together in *Xenopus* oocytes they form an ACh receptor that preferentially allows Ca^{2+} to permeate. In fact, it is about 10 times more permeable to Ca^{2+} than to Na^+ (Weisstaub et al., 2002). The key to the puzzle of hyperpolarization produced by inward current is the presence of Ca^{2+}-activated K^+ channels in close proximity to the ACh receptors. These channels belong to the SK2 family of potassium channels, which are sensitive to micromolar concentrations of Ca^{2+} and insensitive to voltage. That they lie in very close proximity to the ACh channels was demonstrated by the observation that intracellular injection of BAPTA, a calcium chelator with fast kinetics, could prevent activation of the K^+ channels, while injection of EGTA, a calcium chelator with similar affinity but slow kinetics, could not block their activation. Once activated, these Ca^{2+}-activated K^+ channels remain open for a long time, due to the time required to bring intracellular Ca^{2+} down below the concentration that activates them (Fuchs and Murrow 1992; Rohmann et al. 2015).

The net effect of efferent synaptic activity on OHCs is to counteract the depolarizing effect of mechanical activation, thereby inhibiting shortening and reducing their amplifying effect on IHCs. Direct experiments to demonstrate this sequence of events have remained elusive, however, due to the technical difficulty of making electrical recordings from OHCs in an intact cochlea preparation. In the future, this may be an area amenable to the use of voltage-sensitive dyes rather than microelectrode recording.

The effect of efferent modulation on IHCs is more clear-cut; hyperpolarization reduces transmitter release at the afferent synapse, allowing the central auditory system to attenuate or filter out those sound frequencies. This can improve detection of specific sound frequencies against background noise (the "cocktail party" effect) and also protects the cochlea from acoustic trauma (May and McQuone 1995; Lauer and May 2011; Fuente 2015). Developmentally, cholinergic efferents synapse onto both IHCs and OHCs prior to the onset of hearing (Simmons 2002). Efferent synapses develop first on IHCs, where they are initially excitatory due to an absence of Ca^{2+}-activated K^+ channels (SK channels); efferent synapses appear several days later (P6–P8) on OHCs (Roux et al. 2011). There are also differences in the distribution of Ca^2-activated K^+ channels on OHCs; OHCs in the high frequency region have a higher density of large-conductance BK channels, which have faster kinetics (Rohmann et al. 2015).

What, then, does efferent modulation contribute to hearing in non-mammalian vertebrates? The answer seems to be a combination of *increased* overall sensitivity to sound volume and *decreased* sensitivity to tone. This was initially demonstrated by stimulating efferent axons while recording the electrical responses to pure tone acoustic stimuli in hair cells of the turtle cochlea (Art et al. 1985). This experimental paradigm allowed identification of the characteristic resonant frequency of the hair cell. Stimulation of the efferent axons strongly inhibited the hair cell response to tones near its characteristic frequency, as would be expected if the increased conductance to K^+ acted to lower the impedance of the resonant components (voltage-gated Ca^{2+} channels, Ca^{2+}- and voltage-gated K^+ channels). At the same time, however, the hair cell response to lower frequencies was enhanced, but in a flat, frequency-independent manner. This can be explained by an overall *increase* in the cell input resistance, as the hyperpolarization induced by the efferent synapse caused closure of voltage-gated K^+ and voltage-gated Ca^{2+} channels that were open in the resting cell (Fuchs and Parsons 2006).

In contrast to the inhibitory effects of efferent modulation in the auditory system, efferent modulation of vestibular hair cells has both excitatory and inhibitory effects (Jordan et al. 2013). Calyx-bearing afferents (similar to type I cells) are excited by efferent fibers, which synapse postsynpatically onto the afferent nerve ending (Holt et al. 2015). Bouton afferents (similar to type II cells) receive both presynaptic efferent innervation (directly onto the hair

cell) and postsynaptic innervation onto the primary afferent. Efferent stimulation of bouton afferent cells is initially inhibitory, followed by an extended post-inhibitory excitation (Holt et al. 2006). As in the cochlea, acetylcholine is the neurotransmitter and fast synaptic effects are mediated by alpha-9 containing ACh receptors, with inhibitory effects mediated by SK Ca^{2+}-activated K^+ channels (Parks et al. 2017). In addition, muscarinic acetylcholine receptors mediate a slower excitation in calyx-bearing afferents, probably by inhibition of an M-current (Holt et al. 2017).

Evolution of hair cells

Are hair cells a vertebrate innovation? Or do homologous cells exist among invertebrates? This has been an active topic of research and discussion, and has been reviewed in depth elsewhere (Coffin et al. 2004; Burighel et al. 2011). While many invertebrates have mechanosensitive cells bearing cilia and/or microvilli, it has been difficult to identify candidate homologs to vertebrate hair cells. Mechanosensory cells on the tentacles of the sea anemone *Nematostella vectensis* have many interesting properties, including bundles of actin-filled stereocilia bound together by Ca^+-dependent links (Tang and Watson 2014; Menard and Watson 2017). Closer to home in Chordata, Manni and colleagues have described mechanosensitive coronal cells on the oral (incurrent) siphon of tunicates that satisfy multiple criteria for homology with vertebrate hair cells (Caicci et al. 2007; Rigon et al. 2013). These characters include embryological development from placodes, differentiation as secondary sensory cells which synapse onto primary afferents and also receive efferent innervation, and an arrangement of microvilli or stereovilli with graded length, and one or two true cilia, usually located eccentrically (Caicci et al. 2007).

Summary and future directions

Great progress has been made in our understanding of sensory hair cell structure and function. We have a functional understanding of cochlear amplification in mammals and electrical tuning of hair cells in non-mammals, both of which contribute strongly to frequency sensitivity. The motor protein for outer hair cell shortening has been identified, and at least part of the mechanosensitive transduction channel has also been identified. Still, many questions remain about outer hair cell function and there are many gaps in our basic knowledge of hair cell structure. Hair cells should continue to attract basic researchers for many decades to come, and will no doubt repay that curiosity with many experimental insights.

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