Chapter

# Signal Transmission by Auditory and Vestibular Hair Cells

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## Abstract

We interact with the world around us by sensing a vast array of inputs and translating them into signals that can be interpreted by the brain. We have evolved many sensory receptors, each uniquely specialised to detect diverse stimuli. The hair cells are sensory receptors, initially developed to provide a sense of body position and movement, but later adapted to sense minute pressure waves in the environment that are perceived as sounds. As such, hair cells bestow a sense of hearing and balance, which are major advantages for survival. Mammals have four different types of hair cell, two of which are dedicated to hearing, the inner and outer hair cells, and the other two to balance, the type-I and type-II hair cells. While all hair cells employ common mechanisms to detect and relay signals from sound or motion, they also have unique attributes that specialise them for a specific functional role. In this chapter we describe the process of signal transmission in mammalian auditory and vestibular hair cells. Since mammalian hair cells do not regenerate, their loss results in permanent auditory or vestibular deficit. Efforts to regenerate or repair malfunctioning hair cells have recently intensified, mainly through gene, stem-cell and molecular therapy.

**Keywords:** hair cell, cochlea, vestibular, ion channel, ribbon synapse, stem cell, gene therapy

## 1. Introduction

The inner ear of vertebrates houses the auditory and the vestibular systems. Hearing and balance are key senses that allow humans and other vertebrates to acquire important information from the surrounding environment and to detect and compensate for head motion. The receptors responsible for these sensory functions are the hair cells. The defining feature of hair cells is the presence of the hair bundle that protrudes from their apical surface (**Figure 1A** and **B**). The hair bundle is composed of many microvilli-like structures, termed stereocilia, organised in rows of increasing height. The individual stereocilia are connected to one another by different types of membranous linkages, which ensure that the whole hair bundle moves as one functional unit in response to sensory stimulation. One of these links is the tip-link that connects the tip of each shorter stereocilia to the side of the taller adjacent stereocilia (**Figure 1C**). Tip links are composed of cadherin 23 at the upper end, forming the insertion point on the taller stereocilia, and protocadherin 15 at the lower end that connects to the tip of the shorter stereocilia [2] (**Figure 1D**). The lower end of

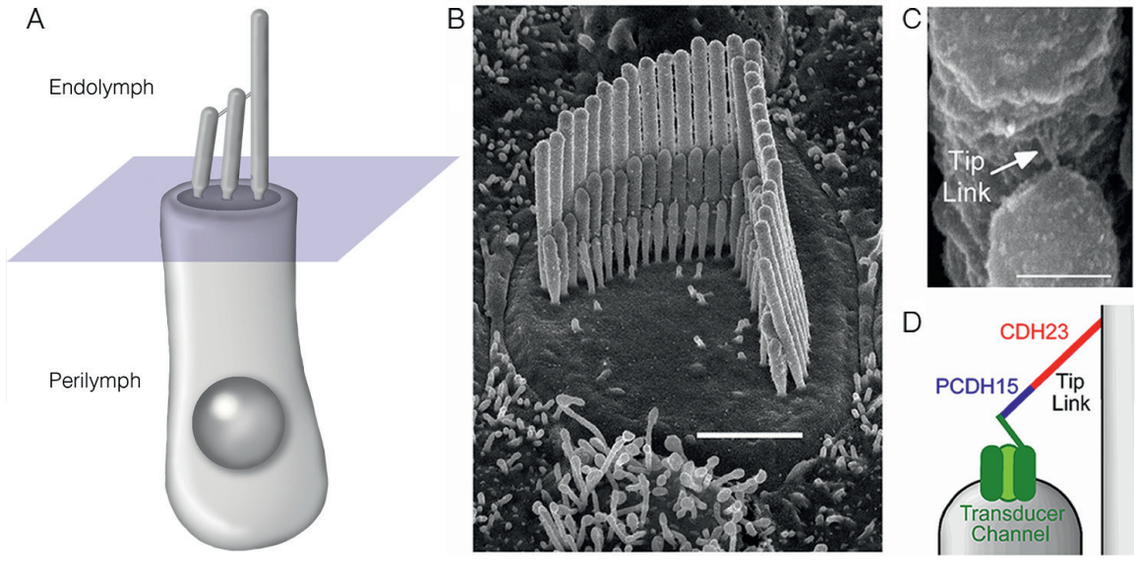


**Figure 1.**
*Hair cell morphology. (A) Cartoon of a generic hair cell. (B) Scanning electron microscope (SEM) image showing the structure of the hair bundle from a cochlear outer hair cell, with the characteristic staircase structure composed of rows of stereocilia of decreasing height. Scale bar: 2 μm. (C) High magnification SEM of a tip link connecting adjacent stereocilia from a cochlear inner hair cell. Scale bar: 200 nm. (D) Drawing showing the tip link connecting the MET channel to the adjacent taller stereocilia. CDH23, cadherin 23; PCDH15, protocadherin 15. Figure modified with permission from Marcotti [1].*

the tip-link joins to a protein complex containing the mechanosensitive, non-selective cation-permeable, ion channel, the so called mechanoelectrical transducer (MET) channel (**Figure 1D**). The best candidate for the MET channel pore is the transmembrane channel-like 1 (TMC1) protein, which has ten transmembrane domains [3, 4]. Mutations of TMC1 results in deafness [5]. Notably, persistent TMC2 expression in vestibular hair cells (TMC2 is only expressed during development in cochlear hair cells) may preserve vestibular function in humans with hearing loss caused by TMC1 mutations [6].

The structural polarisation of the stereocilia gives the hair bundle its axis of mechanical sensitivity. When the sensory-evoked motion of fluid around the hair bundle deflects it towards the taller stereocilia, the tension on the tip links increases and the MET channels open [7]. This allows cations to enter the stereocilia and depolarise the hair cell from its resting membrane potential. Depolarization is graded with the amplitude of the MET current since mature hair cells do not fire action potentials. Deflection of the hair bundle in the opposite direction reduces the tension in the tip links and the MET channels close. In the absence of sensory input, the hair bundle is stationary in its resting position. At rest, there is a slight tension on the tip links that opens a proportion of the MET channels resulting in an inward resting transducer current, which depolarises the resting membrane potential of the hair cells and drives a resting discharge of action potentials in the primary sensory neurons [8, 9].

A crucial feature for hair cell function is the tight separation of the fluid surrounding the apical hair bundle from that surrounding the basolateral cell body (**Figure 1A**). In mammals, the hair bundle is bathed in endolymph, a unique extracellular solution, with a high $K^+$ (157 mM), low $Na^+$ (1 mM) and very low $Ca^{2+}$ concentration (20–40 μM) [10, 11]. The hair cell body is surrounded by perilymph, a normal extracellular solution with low $K^+$ (4 mM) and high $Na^+$ (148 mM) and $Ca^{2+}$ (1.3 mM) concentrations. Potassium is actively secreted into the endolymph by specialised non-sensory epithelial cells found in both the vestibular organs, the

vestibular dark cells, and in the *scala media* of the cochlea, the marginal cells of the *stria vascularis* [12]. In the cochlea, this creates a large electrical potential difference of 80–90 mV between the endolymph and perilymph, called the endocochlear potential (EP) [13]; in vestibular organs, the electrical potential difference is much smaller: 1–11 mV ([14] for a recent review see [15]). Since the hair cell resting potential is around −60 mV, there is a large electrical driving force for $K^+$ entry into the hair cells, via the MET channels, of around 150 mV in the cochlea and 70 mV in the vestibular apparatus. This scenario not only allows a very efficient depolarisation of the hair cells but also provides a route for cell repolarisation since $K^+$ is able to move out of the cell down a large concentration gradient into the perilymph surrounding its basolateral membrane.

The hair cell receptor potential is shaped by the interplay of the depolarising MET current together with the current through voltage-gated ion channels in the hair cell basolateral membrane (**Figure 2**). Hair cell repolarisation is governed by the exit of $K^+$ ions through different types of voltage-gated $K^+$ channel that differ in terms of their kinetics and voltage sensitivity. The depolarising phase of the receptor potential also activates basolateral voltage-gated $Ca^{2+}$ channels that allow $Ca^{2+}$ entry into the hair cell. The influx of $Ca^{2+}$ triggers the release of the neurotransmitter glutamate from specialised ribbon synapses onto postsynaptic afferent fibres (**Figure 2**). Ribbons are electron-dense presynaptic organelles that tether kinetically distinct pools of synaptic vesicles close to the presynaptic membrane [17–19]. The large pools of vesicles allow hair cells to maintain rapid rates of neurotransmitter release and respond to sensory stimulation over prolonged periods of time [18, 20]. Glutamate binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors on the postsynaptic afferent fibres [21–23], triggering action potential activity that is relayed to the brain.

The general process of signal transduction and transmission described above is similar for all hair cells, but mammalian auditory and vestibular hair cells have evolved unique mechanisms and attributes, over hundreds of millions of years, that specialise them to perform specific functional roles. There are four types of hair cell in the mammalian inner ear (**Figure 2**): the inner hair cells (IHCs) and outer hair cells (OHCs) in the cochlea, and the type-I and type-II hair cells in the vestibular organs, which include the saccule, the utricle, and the semicircular canals. In the cochlea, the IHCs are the main sensory receptors whose role is to convert acoustic information into electrical activity and relay it to the spiral ganglion neurons (SGNs), the primary sensory neurons. The OHCs, on the other hand, respond to the same stimulation by changing their length, a unique property called electromotility [24]. So rather than being typical sensory hair cells, the cochlear OHCs form the so called "cochlear amplifier" where their main function is to amplify and fine-tune sound-evoked input to the IHCs, increasing hearing sensitivity and frequency discrimination [7].

In contrast to the auditory hair cells, both the type-I and type-II hair cells in the vestibular system have a sensory role. Type-II hair cells are cylindrically shaped and are contacted by several afferent fibres, similar to auditory IHCs (**Figure 2**). Type-I hair cells have a distinguishing flask-shaped appearance, and their basolateral membrane is almost completely enveloped by a single giant calyx-like expansion formed by a single afferent nerve terminal (**Figure 2**). Type-I hair cells are only present in amniotes, and their appearance with evolution has been associated with the transition to life on dry land and rapid head movements [25]. A lot of progress has been made in understanding the intimate functional and molecular mechanisms of hair cell function using a combination of genetics, structural biology, and electrophysiology. In this

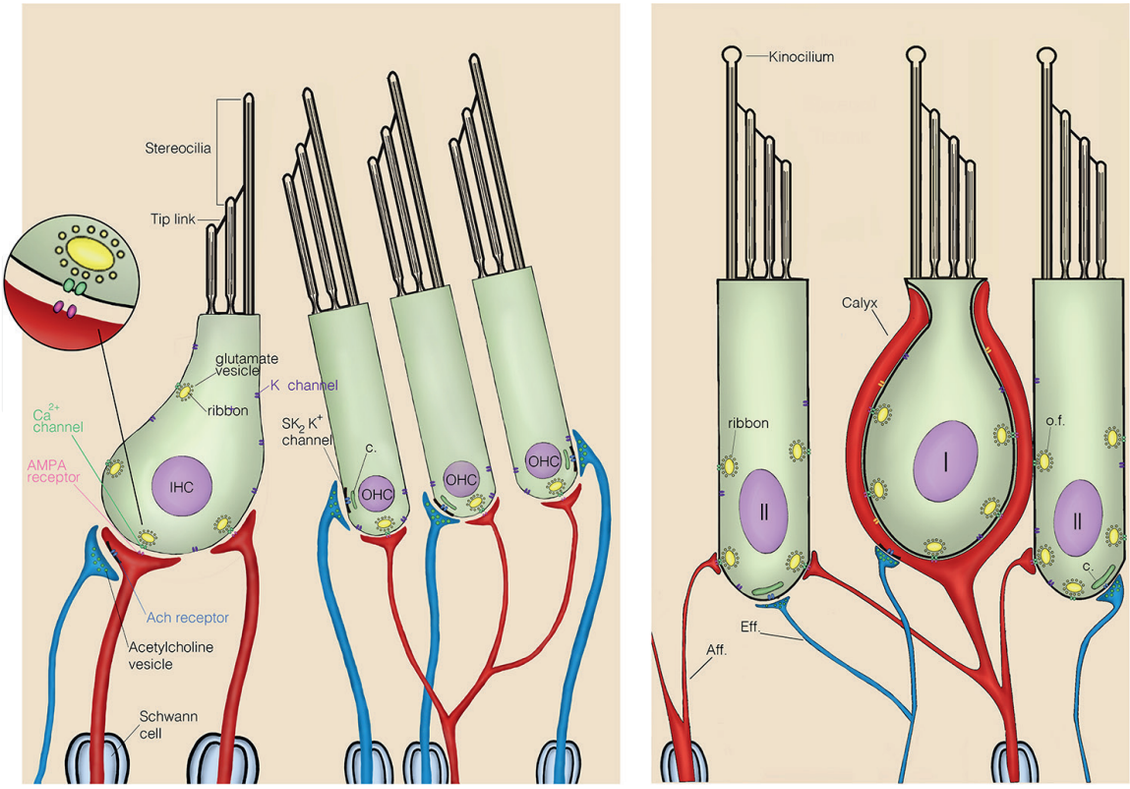


**Figure 2.**
*Auditory and vestibular hair cells and their innervation in adult mammals. Left: in the cochlea, Bouton afferent nerve terminals (red) occur at the basolateral membrane and face a single ellipsoid synaptic body (ribbon, large yellow oval) per active zone in both IHCs and OHCs – Some terminals are not shown in figure for clarity. Presynaptic vesicles (yellow filled circles) containing the afferent neurotransmitter (glutamate) are shown tethered to the ribbon. Voltage-gated $Ca_V1.3$ $Ca^{2+}$ channels (green) are clustered at the presynaptic membrane, opposed to glutamatergic AMPA receptors (magenta) at the afferent postsynaptic membrane. The zoom shows a presynaptic zone facing the postsynaptic membrane. IHCs only form afferent synapses (5 to 30) with type I (myelinated) spiral ganglion neurons (SGNs), which represent ~95% of all SGNs. OHCs form afferent synapses with the remaining ~5% type II (unmyelinated) SGNs. Each type I SGN only forms a single synaptic contact with a single IHC. By contrast, each type II SGN contacts several OHCs. Efferent Bouton nerve terminals (blue) synapse onto type I SGN boutons in close proximity to IHCs (unmyelinated lateral olivocochlear (LOC) neurons), or directly contact OHCs (myelinated medial olivocochlear (MOC) neurons). Only a single LOC efferent is shown for clarity while, in reality, a rich plexus of endings is formed beneath each IHC. Presynaptic vesicles (green filled circles) containing the efferent neurotransmitter (acetylcholine) are also shown. Cholinergic receptors (blue) facing the efferent terminal are located at the OHC postsynaptic membrane, and at the afferent Bouton contacting the IHC. The efferent synapse onto OHCs is marked by a subsynaptic cistern (c.). right: in the vestibular epithelia, afferent nerve terminals (red) are myelinated and are classified as calyx-only if they only contact type-I HCs, dimorphic if they contact both type-I and type-II HCs, and Bouton-only if they only contact type-II HCs. Each Bouton afferent terminal faces a single ribbon, whereas each calyx is opposed to several (7–20) ribbons. Efferent neurons (blue) originating in the brainstem directly contact several type-II HCs and may also contact calyces and boutons. An outer face (o.f.) synapse between the type-II hair cell and the calyx is also shown [16], whose function remains to be elucidated. Note that a single eccentric cilium called kinocilium, which is found adjacent to the longest stereocilia row, is present in mature vestibular hair cells but not in cochlear hair cells which lose it during maturation. The kinocilium is likely involved in the morphogenesis of the hair bundle during embryonic development, while the reason for its persistence in mature vestibular hair cells is unknown.*

chapter, we describe the current understanding of signal processing and transmission by the different mammalian hair cell types.

The differentiation of auditory or vestibular hair cells from common cell precursors is guided by an extensive array of molecules, expressed with a highly ordered spatiotemporal pattern, which is only recently beginning to be understood [26–29]. The cost of such an elaborate program of differentiation is that the mammalian inner ear is unable to replace hair cells loss due to, for example,

damage, genetic mutation or ageing. More and more gene mutations are becoming identified that produce, or cause predisposition to, inner ear disorders due to hair cell malfunction or even death [30, 31]. At the end of this chapter, we look at the different strategies that are being developed as potential therapies to regenerate hair cells or to restore their function.

## 2. The inner ear

### 2.1 Signal processing in the cochlea

The mammalian auditory system can sense and transmit different acoustic features such as sound frequency, intensity, timing and duration, all of which have vast dynamic ranges. Humans, for example, have a hearing frequency range spanning three orders of magnitude, from 20 Hz to 20 kHz or approximately 10 octaves, and can detect sound intensity differences of over 12 orders of magnitude, up to 120 dB. We can discriminate small sound level differences with nearly constant acuity (~1 dB) across almost the entire range [32, 33]. In order to achieve this, the mammalian cochlea has evolved a number of different strategies. The cochlea has a spiralled structure and contains around 3500 IHCs and three times as many OHCs. Sound enters the cochlea via the ear drum and the ossicles in the middle ear. The cochlea is tonotopically organised, such that hair cells at the base of the spiral respond best to high-frequency sound, and cells at the apex respond best to the lowest frequencies. Cochlear tonotopy is primarily imposed by the mechanical properties of the basilar membrane upon which the hair cells reside, which is wider and more flexible at the apical low frequency end and narrower and stiffer at the basal high frequency end [34]. Sound causes a wave that travels along the basilar membrane with the largest oscillation occurring at the base of the cochlea for high frequency sounds and towards the apex for lower frequencies [13]. The movement produced by the oscillation of the basilar membrane causes the hair bundles of the IHCs and OHCs to move back and forth at the sound frequency, opening and closing the MET channels, creating a receptor potential in the hair cells. The sinusoidal change in receptor potential modulates neurotransmitter exocytosis in IHCs and electromotility in the OHCs [35, 36]. The combined electromotile activity of the three rows of OHCs amplifies the sound-induced motion of the basilar membrane. Cochlear amplification ensures that each IHC is sharply tuned to a narrow sound frequency band, known as its *characteristic frequency* (CF) (for a review see [37]).

The tonotopic organisation of the cochlea translates the frequency code of sound into a place code. Each IHC along the cochlea represents a narrowly tuned frequency channel, like the keys on a piano, that form a tonotopic frequency map. This map is preserved and represented throughout the auditory pathway, all the way to the cortex. A sound frequency is therefore represented by the relative position of the active IHC, or neurons, within the tonotopic map, rather than being encoded by the receptor potential or neuronal firing rates; although this does also occur for frequencies below a few KHz (see next section). While sound frequency is represented by the tonotopic map and split among all the IHCs within each cochlea, the duration, timing and entire intensity range has to be encoded by neurotransmitter release from every IHC and distributed among the afferent nerve fibres of SGNs, which are estimated to be about 35,000–50,000 [38]. The functional mechanisms underlying IHC sound processing are described in the following section.

### 2.1.1 Cochlear inner hair cells

The IHCs convert the sound-induced motion of the hair bundle into a receptor potential that drives neurotransmitter release onto auditory afferent neurons. For frequencies below ~2 kHz, the IHC receptor potential follows the MET current with cycles of depolarisation and hyperpolarisation at the stimulating sound frequency. Therefore, the receptor potential of low frequency IHCs is dominated by an alternating current 'AC' component that 'phase-locks' to the sound frequency [39, 40]. The magnitude of the AC component is proportional to the size of the MET current, which is greater for louder sounds [39, 41]. Above a few kilohertz, the filtering properties of the IHC membrane prevents the receptor potential from phase-locking. As such, high frequency IHCs respond to sound with a depolarising shift in membrane potential, a direct current 'DC' component, that is sustained for the duration of the sound. As for the AC component, the magnitude of the DC component is proportional to the loudness [41, 42].

The receptor potentials of low- and high-frequency IHCs are determined by the interaction of the MET current with that through different voltage-dependent $Ca^{2+}$ and $K^+$ channels expressed in their basolateral membrane. About 90% of the $Ca^{2+}$ current in all IHCs is carried by $Ca_V1.3$ $Ca^{2+}$ channels [43, 44]. Consistent with a crucial role of $Ca_V1.3$ $Ca^{2+}$ channels in IHC signal transmission, deletion of $Ca_V1.3$ in mice [44, 45] or loss of its function in humans [46] produces deafness. The $Ca^{2+}$ channels provide the inward $Ca^{2+}$ current that is vital for IHC depolarization as well as for triggering exocytosis (see below). Adult mouse IHCs also have several different $K^+$ channel types that provide the outward $K^+$ current required for cell repolarisation and for setting the resting membrane potential. There is a voltage-dependent $K^+$ current characterised by slow activation kinetics ($I_{Ks}$), a negatively activating delayed-rectifier $K^+$ current ($I_{K,n}$), and a large voltage- and $Ca^{2+}$-dependent $K^+$ current characterised by fast activation kinetics ($I_{Kf}$) [47–49]. Several $K^+$ channel subunits have been identified so far, which account for the above $K^+$ currents: Kv1.8, Kv7.4, Kv11.1, and Kv12.1 for $I_{Ks}$, $KCNQ_4$ (Kv7.4) for $I_{K,n}$, and $BK_{Ca}$ channels for $I_{Kf}$ [50]. Consistent with the different receptor potential responses of low- and high-frequency cells, tonotopic differences in the biophysical characteristics of IHCs combine to optimise IHC responses to acoustic stimuli of different frequency [51]. Low frequency IHCs have a large MET channel resting open probability (>40% open at rest in physiological conditions) compared to that in high frequency cells (<10%), resulting in the former having a more depolarised resting potential [51]. Low frequency IHCs also have a different complement of voltage-gated $K^+$ channels compared to high-frequency cells [51]. For low frequency IHCs, the depolarised resting potential activates a large proportion of the basolateral membrane currents, to increase the speed and accuracy of the frequency-following component of the receptor potential [50, 51]. For high frequency IHCs, the more hyperpolarised resting potential and smaller proportion of currents active at rest allow the responses of these cells to summate into a DC membrane potential shift proportional to stimulus intensity [51]. Therefore, both low and high frequency IHCs are intrinsically tuned to accurately represent the main component of the cells' *in vivo* receptor potential, showing a preference for either timing (AC) or intensity (DC) coding, respectively. While low frequency IHCs respond rapidly and accurately to phasic stimuli up to the phase-locking limit, high frequency cells respond with clearly defined graded shifts in membrane potential over an extended dynamic range.

The specialisation of low and high frequency IHCs for either response timing or graded accuracy, respectively, is likely to be related to the main mechanisms used for localising low or high frequency sounds. In mammals, low frequencies are localised using the inter-aural timing difference (ITD) of a sound arriving at the two ears, by the CNS comparing the differences in timing of the phase-locked activity in auditory afferents [52]. High frequency sounds are conversely localised using the inter-aural level difference (ILD) of a sound between the two ears, by comparing differences in the level of activity in the afferents [53]. Therefore, the IHC biophysical properties are best suited to maximise the accuracy of response-timing in low frequency cells and response-level in high frequency cells, allowing the detection of ITDs as small as 10 ms and ILDs of only 1–2 dB [54]. As well as the differences in their biophysical properties, IHCs also show tonotopic differences in the number, organisation and function of their ribbon synapses that favour transmission of the intrinsic tuning for either phasic or graded receptor potentials (see below).

All of the acoustic information encoded in the IHC receptor potential, including timing, intensity and duration, has to be faithfully encoded by neurotransmitter release at the IHC ribbon synapses into activity in the SGNs. Each IHC has between 5 and 30 presynaptic active zones [55] with a specialised synaptic ribbon and post-synaptic afferent bouton from an unbranched type-I SGN [56, 57] (**Figure 2**). The individual ribbon synapses within each IHC have different activation ranges, presumably to extend the range of stimulus intensities transmitted by each IHC [58–61]. As well as differences between individual synapses there are also tonotopic differences in the overall synaptic function of IHCs, which could represent a sound frequency-dependent tuning of exocytosis that correlates with the specialisation for phasic or graded receptor potentials described above. In the following paragraphs we will focus on these tonotopic specialisations and how they relate to the different receptor characteristics of low and high frequency IHCs.

The IHC synaptic ribbons are electron-dense presynaptic organelles, composed mainly of the protein ribeye [62]. The function of the synaptic ribbon is to tether kinetically distinct pools of synaptic vesicles close to the release sites to provide a rapid and relatively inexhaustible release of the neurotransmitter glutamate in response to acoustic stimulation (for recent reviews see [19, 63, 64]). The vesicles attached to the base of the ribbon and docked at the presynaptic active zones form the readily releasable pool (RRP), consisting of a few hundred vesicles per IHC [17–19], and are the first to be released following stimulation. The ribbon associated vesicles that are further away from the release sites together form a larger secondarily releasable pool (SRP), which is believed to refill the RRP once it has become depleted to maintain relatively high rates of exocytosis for long periods of stimulation [19, 63, 64].

As mentioned above, exocytosis of glutamate-containing synaptic vesicles at IHC ribbon synapses is triggered by the entry of $Ca^{2+}$ through closely coupled voltage-gated $Ca_V1.3$ $Ca^{2+}$channels in response to cell depolarisation, similar to conventional synapses. These channels are suited for driving neurotransmitter release at sensory synapses since they activate at around, or below, the IHCs estimated resting potential (about −60 mV: [51, 65, 66]). $Ca_V1.3$ channels also show rapid activation kinetics (sub-millisecond), which is required for precise timing [65–67], and little voltage- or $Ca^{2+}$-dependent inactivation, allowing them to drive prolonged or continuous neurotransmission [45, 68]. Unlike conventional synapses, however, many of the established synaptic proteins have not been found at ribbon synapses, such as the SNARE proteins [69] nor the traditional $Ca^{2+}$ sensors of exocytosis, synaptotagmin

1 and 2 [64, 70]. Instead, IHC ribbon synapses are likely to rely on the function of the ribbon itself, along with the structural proteins bassoon and piccolino (for reviews see [19, 63, 64]) and the multifunctional role of the $Ca^{2+}$ binding protein otoferlin [71–73]. Otoferlin has multiple $Ca^{2+}$ binding domains and is considered to be the main $Ca^{2+}$ sensor for synaptic vesicle exocytosis at IHCs ribbon synapses with multiple roles in the synaptic vesicle cycle, including vesicle priming, docking, fusion, vesicle pool replenishment and endocytosis [19, 20, 71, 72]. Consistent with the primary role of otoferlin in IHCs, *otoferlin* knockout mice are deaf, and mutations in human otoferlin causes a nonsyndromic form of deafness [71, 74].

As well as otoferlin, the $Ca^{2+}$ dependence of neurotransmitter release at IHC ribbon synapses has been shown to be dependent on the unconventional synaptotagmin isoform, synaptotagmin 4 (Syt4) [75]. Synaptotagmin 4 is unusual since it does not bind $Ca^{2+}$ in the normal $Ca^{2+}$ sensing domain for exocytosis [76] and, as such, is believed to work together with otoferlin to decrease the overall $Ca^{2+}$ dependence of exocytosis in mature IHCs [75]. The $Ca^{2+}$ dependence of IHC synaptic vesicle release determines its operating range and sensitivity to both small and large fluctuations in the cell receptor potential. This important property has been studied in IHCs from different cochlear regions using patch clamp to record $Ca^{2+}$ currents and changes in cell membrane capacitance ($\Delta C_m$) that are indicative of exocytosis from the cells complement of ribbon synapses [75, 77–82]. In mature IHCs, neurotransmitter release is graded with cell membrane depolarization and, apart from very low-frequency IHCs (see below), is linearly dependent on $Ca^{2+}$ entry [78, 79, 83]. Such a linear relation seems to be a feature of many sensory synapses where graded amounts of neurotransmitter release are required [19]. The functional significance of the linear relation is likely to be that it extends the dynamic range of sound intensity discrimination by facilitating exocytosis at low levels, as well as preventing synapse saturation at high levels. While this is true for high frequency IHCs, such as those in the mouse (sensitive to sounds above a few kHz), very low frequency cells, such as those of the gerbil (CF around 300 Hz) have a high-order exocytotic $Ca^{2+}$ dependence [80, 81]. Such supralinear relation is thought to emphasise the phasic component of the receptor potential to accurately localise very low frequency sounds [51, 81].

While the mechanism responsible for the tonotopic differences in IHC $Ca^{2+}$ dependence remains unknown, the linear relation seems to be correlated with the presence of Syt4, with high frequency mouse and gerbil IHCs showing its expression whereas it is absent from low frequency gerbil IHCs [75]. This suggests that the $Ca^{2+}$ dependence could be determined by the intrinsic properties of the $Ca^{2+}$ sensor, which is likely to be otoferlin, with or without Syt4. Alternatively, such differences could also arise from a variation in the topographic coupling of $Ca^{2+}$ channels and vesicle release sites [55, 78]. It has been suggested that a linear exocytotic $Ca^{2+}$ dependence could arise from a very close 'nanodomain' coupling between the $Ca^{2+}$ channels and vesicle release sites [78, 84], whereby the opening of a $Ca^{2+}$ channel within a few tens of nanometres from a vesicle, provides enough $Ca^{2+}$ to saturate the $Ca^{2+}$ sensor and trigger vesicle fusion [66, 78]. In this situation, an apparently linear overall relation is created by the one-to-one association of $Ca^{2+}$ channel openings and vesicle release, even though the exocytotic $Ca^{2+}$ sensor has an intrinsically high-order $Ca^{2+}$ dependence [77, 84]. Direct evidence for the nanodomain coupling between $Ca^{2+}$ channels and vesicle release sites in mouse IHCs has come from the finding that the rapidly binding $Ca^{2+}$ chelator BAPTA inhibited exocytosis more than the slowly acting EGTA [82]. However, recent experiments using EGTA showed that exocytosis could be uncoupled from $Ca^{2+}$ entry in high frequency IHCs (CF > 2 kHz) but was more

resistant in low frequency cells (CF < 2 kHz) [81]. This implies that high frequency IHCs have a looser 'microdomain'-like coupling whereas low frequency cells have the tighter 'nanodomain' organisation. Therefore, it is likely that the $Ca^{2+}$ dependence of exocytosis is determined by the intrinsic $Ca^{2+}$ binding properties of the $Ca^{2+}$ sensors, as well as the architecture of the ribbon synapses.

The tonotopic differences in the functional characteristics of the IHC synaptic machinery are likely to represent further frequency-specific tuning of the cells to accurately represent the main components of their receptor potential, and optimise the responses of the primary auditory neurons. The high-order exocytotic $Ca^{2+}$ dependence and tight nanodomain coupling of low frequency IHCs, together with their more rapid receptor potential responses (see above), could facilitate the signalling of phase-locked activity up to the highest frequencies possible [51, 81, 85]. By contrast, high frequency IHCs do not follow the frequency component of sound, but instead have to precisely reflect the graded changes in the amplitude and kinetic properties of the macroscopic $Ca^{2+}$ current over a wide dynamic range of sound intensity, which is more in line with a linear $Ca^{2+}$ exocytotic dependence and microdomain coupling involving more than one $Ca^{2+}$ channel [85]. The microdomain coupling of exocytosis is known to enhance the "signal-to-noise" ratio of transmission, by reducing the noise associated with the stochastic $Ca^{2+}$ channel openings [86]. Therefore, mature IHC synapses seem to have developed remarkable frequency-dependent tuning. On one hand low frequency cells show submillisecond encoding of receptor potential fluctuations for accurate phase-locking, while on the other hand, high frequency cells show precise signalling of graded receptor potentials.

A nanodomain coupling of $Ca^{2+}$ channels and synaptic release sites in IHCs that show phase-locked activity to low frequency stimulation has also been suggested from recordings of postsynaptic activity from individual afferent fibres or bouton terminals. The close nanodomain coupling has been used to explain how postsynaptic activity is phase-locked to a particular time point (phase) of the sinusoidal stimulus, independent of its magnitude or intensity [87–90]. In a nanodomain scenario, exocytosis is governed by the properties of the single closely coupled $Ca^{2+}$ channel, such that the increasing channel open probability with depolarization (sound intensity) would similarly increase the fusion probability of the "competent" (nearby) vesicle, resulting in an action potential at the afferent fibre [85]. This scenario could explain why the frequency, but not the amplitude, of EPSCs increases with IHC depolarization [22, 91–93].

It is possible that heterogeneity between individual synapses within the same IHC, in terms of exocytotic $Ca^{2+}$ sensitivity [94], or the size and voltage-dependence of the $Ca^{2+}$ influx [55], could influence the tonotopic variation in the cell's synaptic characteristics. However, differences in the properties of neurotransmitter release among individual synapses, as a function of their tonotopic position along the cochlea, remains unknown. Tonotopic differences have been observed in the spontaneous firing rate of afferent fibres in the gerbil that generally have a higher frequency at the apex than those at base [95], which is likely to reflect the underlying synaptic ribbon function.

### *2.1.2 Cochlear outer hair cells*

Cochlear amplification results from OHC electromotility, whereby the cells change length in response to fluctuations in their receptor potential [37]. For cochlear amplification to work, the motion of the OHCs must follow the stimulating sound on

a cycle-by-cycle basis. Therefore, to drive electromotility throughout the entire auditory range, the OHC receptor potential must have an AC component able to accurately follow the MET current up to the highest audible sound frequencies.

The OHCs have, therefore, evolved biophysical specialisations that enhance response speed, which are similar to those described for low-frequency IHCs (see above) and are required to facilitate accurate phase-locking up to a few kHz. The resting open probability of the MET channels in OHCs is about 50% throughout the length of the cochlea when experiments are performed using endolymphatic-like $Ca^{2+}$concentration [96]. This means that displacement of the stereocilia by the acoustic vibration results in a symmetric sinusoidal voltage excursion of the OHC receptor potential around its resting level. The large resting MET current also means that OHCs have a depolarised resting membrane potential of around −40 mV *in vivo*, which would activate a substantial proportion of the cell's voltage-gated channels.

The predominant voltage-gated $K^+$ channel expressed in OHCs is $KCNQ_4$ (Kv7.4; [97]) with mutations in $KCNQ_4$ producing deafness [98]. $KCNQ_4$ channels carry the large delayed rectifier $K^+$ current $I_{K,n}$, defined by its very negative voltage activation range [99]. There is a tonotopic gradient in the size of $I_{K,n}$ with high frequency OHCs having a larger current than that present in low frequency cells [96, 100]. The depolarised resting potential of OHCs would activate over half of the $KCNQ_4$ channels, resulting in high-frequency cells having a larger resting $K^+$ current (and smaller membrane resistance) than low-frequency cells. There is also a tonotopic difference in the overall size of the OHCs, such that high frequency cells are smaller with lower cell membrane capacitance compared to low frequency cells [43, 96, 100]. Since the cell membrane time constant is determined by the product of cell membrane resistance and membrane capacitance, the smaller values for both in high frequency OHCs greatly increase the speed at which the cells can respond. In fact, the time constant of the OHC membrane was found to be appropriate for allowing voltage responses at their CF as a function of position along the cochlea's tonotopic axis [96]. Therefore, OHCs show frequency-dependent differences in their biophysical properties that tune their voltage responses to CF, with high frequency cells being faster than low frequency cells. The tuning of OHCs is opposite to that seen for IHCs, where very low frequency cells showed the most rapid responses in order to phase-lock to sound up to only a few kHz.

The OHCs also contain $Ca_V1.3$ voltage-gated $Ca^{2+}$ channels [101, 102] that drive the exocytosis of glutamate from their ribbon synapses onto the type-II afferent fibres innervating them. The size of the $Ca^{2+}$ current (measured as a barium current in some studies) in immature and mature OHCs is much smaller than that present in IHCs of comparative ages [75, 102–104], consistent with the presence of fewer synaptic ribbons and afferent terminals on these cells [105]. The voltage dependence of the $Ca^{2+}$ current in mature OHCs was found to be shifted by around 10 mV in the depolarised direction compared to that in IHCs [103], which would have implications on the activity of the Type-II SGNs (discussed below).

The OHC receptor potential is converted into electromotility by the motor protein prestin [106]. The OHCs are unique in that they are the only cells known to be endowed with somatic electromotility. Prestin is encoded by the *SLC26A5* gene, a member of the SLC26 anion transporter family [106] which has, however, lost its transport function [107]. Prestin is packed in the OHC lateral membrane at an amazingly high density of over $10{,}000/\mu m^2$ [108]. In response to the sinusoidal depolarization produced by the MET current during acoustic stimulation, prestin acts as a reverse piezoelectric, by mechanically changing its conformation as a function

of voltage (see [37]). As a result, OHC depolarization or hyperpolarisation shortens or lengthens the OHC, respectively, by a few μm. Since the tips of the OHC stereocilia are firmly attached to the underside of the tectorial membrane, unlike those of IHCs, the change in OHC length amplifies the sound-induced oscillation of the local cochlear partition, including the basilar membrane. The result is a stronger mechanical stimulus to the adjacent IHCs, which increases their sensitivity (by as much as 40–60 dB in mice; [109]) and sharpness of frequency tuning. The importance of prestin is also underlined by the finding that mutations produce deafness in humans [110].

Consistent with the local amplifying role of OHCs, they are only contacted by around 5% of all the SGNs in the cochlea, the Type II afferents, which are characterised by thin, unmyelinated axons (**Figure 2**). Each Type II afferent extends hundreds of mm along the cochlear duct to contact several OHCs (up to around 30; [111]). Each OHC, in turn, contacts two to three afferent fibres of Type II SGNs [112]. Exocytosis in OHCs occurs at ribbon synapses, like those of IHCs, with each mature OHC containing only very few ribbons (around 1–4 ribbons per cell: [105]). The presynaptic function of OHCs has been investigated with capacitance measurements only in immature cells and$\Delta$ $C_m$ has been found to be much smaller than that of IHCs [75], consistent with their fewer synaptic ribbons. Postsynaptic recordings have shown that, unlike the Type I fibres that contact the IHCs, Type II afferent fibres seem to be activated only when all their OHC inputs are active simultaneously [113, 114]. Such a situation would only by be caused by sounds loud enough to cause acoustic trauma [113, 115, 116], and as such the function of Type II fibres is likely to be related to sensing damage or nociception in the cochlea. Recently, however, it has been shown that Type-II afferents can be activated by non-damaging, loud sounds [117]. The more depolarised activation threshold for the $Ca^{2+}$ current in mature OHCs [103], could be compensated by the much more depolarised *in vivo* resting potential of the OHCs to increase the probability that synapses are simultaneously active. These findings point to a more active role for Type II afferents in auditory signal processing.

Type II activation could be involved in transmitting information to the CNS to activate feedback efferent pathways to reduce cochlear amplification and avoid/limit further damage to the cochlea. The efferent input to the OHC constitutes the majority of their innervation (**Figure 2**). The efferent fibres originate from the medial olivocochlear (MOC) nucleus in the auditory brainstem [118]. Efferent fibres release the neurotransmitter acetylcholine (ACh), the role of which is to inhibit OHC electromotility and hence reduce the mechanical amplification of the cochlear partition [119]. OHC inhibition is achieved because ACh, by promoting $Ca^{2+}$ influx through α9α10 nicotinic acetylcholine receptors [120, 121], leads to the opening of co-localised small conductance $Ca^{2+}$-activated $K^+$ channels ($SK_2$: [122]). The efflux of $K^+$ results in OHC hyperpolarisation. Intracellular $Ca^{2+}$ diffusion away from $SK_2$ channels is limited by a thin near-membrane cistern that is co-extensive with the efferent terminal contact (**Figure 2**).

### 2.2 Signal processing in the vestibular organs

In mammals there are five vestibular sensory organs: the two otolith organs, comprising the perpendicularly-arranged utricle and sacculus, and three orthogonally-arranged semicircular canals (horizontal or lateral, anterior, and posterior). The utricle and saccule detect horizontal and vertical linear acceleration, respectively, and contain a sensory epithelium called the *macula*, composed of a flat sheet of sensory

hair cells and supporting cells. The hair cell stereocilia project into the gelatinous otolithic membrane that has otoliths, or ear stones, on its upper surface. The semicircular canals detect angular acceleration in the three spatial dimensions and contain a sensory epithelium that is called the *crista,* which is similar to the *macula* but smaller. In the *crista* the hair cell hair bundles project into the gelatinous cupula. On average, there are around 10,000 vestibular hair cells in each mouse labyrinth, distributed nearly equally between otolith and canal epithelia [123]. As mentioned in the introduction, vestibular hair cells are subdivided into type-I and type-II cells based on their cell body shape and innervation pattern [16, 123–127] (see **Figure 2**). The sensory epithelia in the vestibular organs act as accelerometers, detecting motion due to linear or angular acceleration and gravity. These forces act on the otolithic membrane or the cupula and, combined with the inertia of the endolymph within the vestibular organs, deflects the hair bundles of both type-I and type-II hair cells. This activates vestibular hair cell signal transduction and information is relayed to the brain via the vestibular afferent fibres. Vestibular information is used centrally to drive several motor reflexes. These include the incredibly fast vestibulo-ocular reflex that maintains eye position and gaze stabilisation [128], and those controlling balance and posture [129]. Vestibular information is also used to generate spatial memory, and to support orientation and navigation of an individual in the environment [130, 131]. When vestibular signalling is impaired, disabling pathological conditions arise such as vertigo, nausea, ataxia, an altered perception of self-orientation and oscillopsia.

The vestibular system is considered to be a low-frequency analyser since it provides information about velocity of naturally occurring head movements at frequencies ranging from 0.1 to a few tens of Hz, well below the frequency of acoustic stimuli sensed by cochlear hair cells [132, 133]. Therefore, there is no graded organisation of the hair cells according to their characteristic frequency, as observed in the cochlea [51]. In the vestibular sensory epithelia, hair cells are instead arranged in two different zones. In the *maculae* of the otolithic organs, there is a curved central stripe, called the striola, that shows distinct morpho-functional properties with respect to the remaining outer area of the sensory epithelium, called the extrastriolar region. There is a similar distinction in the *cristae*, with a central zone being equivalent in many ways to the striolar region of the macula, and a peripheral zone that is similar to the extrastriolar region (reviewed in [25]). One major functional difference between the zones relates to the firing activity in the afferent fibres that contact the hair cells. In the striolar and central zones, afferents show highly irregular spike activity and adapting responses to head motion, which are best suited for encoding rapid phasic signals (transient motions; [123]). By contrast, afferents from the extrastriolar and peripheral zones show regular tonic spike trains, suitable for encoding slow movements and sustained stimuli, such as maintained head tilts and gravity [123, 124, 134, 135].

The correlation between hair cell type and the zonal segregation is less clear, since both type-I and type-II hair cells are distributed throughout the vestibular sensory epithelia. There is, however, a zonal difference in the afferent wiring patterns of the hair cells. Hair cells transmit their signal onto three different classes of vestibular afferents. The calyx-only afferents only innervate type-I hair cells, with the calyx almost completely enclosing the basolateral surface of the hair cell, and can surround a single, or multiple, type-I cells. Bouton-only afferents have smaller terminals, similar to those on cochlear hair cells, and branch to innervate several type-II hair cells. Finally, dimorphic afferents represent the majority of vestibular afferents and are branched to form both calyx endings on type-I hair cells and bouton terminals on

type-II hair cells [132, 135, 136] (**Figure 2**). While both calyceal and bouton terminals are found throughout the vestibular sensory epithelium, afferents in central/striolar zones are either calyx-only or dimorphic, such that all irregularly firing afferents receive a proportion of their input from type-I hair cells. Afferents in peripheral/extrastriolar zones are either bouton-only or dimorphic, such that all regularly firing afferents contact at least one type-II hair cell. Therefore, while there is a degree of overlap from the dimorphic afferents, it grossly appears that type-I hair cells are responsible for the rapid phasic component of the afferent response, while type-II hair cells are required for the tonic component.

### *2.2.1 Vestibular type-I and type-II hair cells*

Both type-I and type-II hair cells transduce head motion into a firing activity within the vestibular ganglion neurons. A major goal of vestibular neuroscience is to understand the distinct functional roles of type-I and type-II hair cells. It is thought that type-I hair cells and their encapsulating calyces might have evolved more recently in amniotes (anamniotes only have type-II hair cells) to allow more rapid transmission of information, possibly required for the transition to a land-based life and the acquisition of a head moving independently of the body trunk [25].

The receptor potential in both type-I and type-II vestibular hair cells is driven by the depolarizing current flowing through MET channels following sensory-evoked displacement of the hair bundles [137, 138], as for cochlear hair cells. Hair cell depolarisation then activates voltage-gated $K^+$ channels expressed in their basolateral membrane, the opening of which results in cell repolarisation [139, 140]. While this mechanism is similar to that of cochlear hair cells, the complement of underlying $K^+$ channels differs not only with cochlear hair cells but also between type-I and type-II hair cells. In type-II hair cells, the total outward $K^+$ current is composed of a delayed-rectifying current, an A-type inactivating current, and a $Ca^{2+}$-activated $K^+$ current. Type-II hair cells also express the hyperpolarisation-activated (anomalous rectifying) $K^+$ current and the mixed $Na^+/K^+$ permeable $I_h$ [141]. As well as an $I_h$ current [141], type-I hair cells have a delayed rectifier $K^+$ current component, together with a large outwardly rectifying $K^+$ current, termed $I_{K,L}$, that activates at very hyperpolarised membrane potentials, such that it is about half activated at the hair cell's resting membrane potential of around −70 mV ([142–144]; see [28] for a comparison of ion channels expressed in auditory and vestibular sensory epithelia). The molecular identity of the channel carrying $I_{K,L}$ remains unknown, however, both KCNQ and ether-a-go-go (erg) $K^+$ channel subunits have been suggested to contribute [145]. The substantial activation of $I_{K,L}$ at the resting potential of type-I hair cells means that these cells have a significantly lower resting membrane resistance (typically less than 50 MΩ) compared to type-II hair cells (about 1 GΩ) [142, 143, 146]. Since the speed of the cells' voltage responses is determined by the membrane time constant, the low membrane resistance of type-I hair cells means they have faster but smaller voltage responses compared to type-II hair cells (see [147]). This is consistent with the different functional roles proposed for type-I and type-II hair cells as either rapid phasic receptors or slower graded receptors, respectively.

The transmission of vestibular information from the hair cells takes place at the interface between the cells and the afferent terminals. As for auditory hair cells, this occurs at ribbon synapses present in both types of vestibular hair cell, where the $Ca^{2+}$-dependent release of glutamate triggers an action potential discharge at the postsynaptic terminals [16, 21, 23, 126, 137]. An important difference is that while

each auditory afferent receives the sensory input from a single ribbon, multiple ribbons contribute the sensory input to a single vestibular afferent due to their branching (**Figure 2**). Therefore, each vestibular neuron integrates the information from several type-I and/or type-II hair cells. In the case of the calyx, there is an additional integration of information from the multiple ribbon synapses in each type-I hair cell it surrounds. As well as the quantal release of glutamate, type-I hair cells have been shown to use a non-quantal mechanism of signal transmission that relies on the unique architecture of the postsynaptic calyx [25, 148, 149]. The two modes of vestibular hair cell transmission are described below.

The process of quantal synaptic transmission in both vestibular hair cell types is similar to that described above for IHCs. Both type-I and type-II hair cells have similar numbers of synaptic ribbons, ranging from 7 to 20 per cell [21, 23, 126]. The fusion of synaptic vesicles at the ribbon synapse release sites is triggered by $Ca^{2+}$ influx through $Ca_V1.3$ $Ca^{2+}$ channels, and mediated by the $Ca^{2+}$ sensor of exocytosis otoferlin [21, 23, 139, 150–153]. Mature utricular type-II hair cells have a relatively large RRP and SRP of synaptic vesicles that become recruited following sustained stimulation [153–155]. The large synaptic vesicle pools in type-II cells, which are similar in size to those in mature IHCs, allow them to sustain the transmission of tonic signals. This is important for maintaining head orientation relative to gravity and for encoding slowly varying signals such as low-frequency head movements during walking or running [156]. Mature utricular type-I hair cells, on the other hand, show much less synaptic vesicle exocytosis, with an RRP, which is around an order of magnitude smaller than that in type-II hair cells, and little evidence of an SRP [153]. Although the overall amount of exocytosis differs between type-I and type-II hair cells, the number of vesicles released in the RRP of a mature type-I cell onto a single calyx is likely to be comparable to the amount of RRP vesicles released by type-II cells onto a single afferent terminal [153]. The relatively small degree of exocytosis in mature type-I hair cells is likely to be a developmental adaptation since it is much larger at immature stages [21, 72].

In mature utricular type-II hair cells neurotransmitter release has a high-order $Ca^{2+}$ dependence [153–155], similar to that found in low frequency mature IHCs [81]. The $Ca^{2+}$ dependence of the smaller exocytotic component in mature type-I hair cells remains to be established. A high-order relation in vestibular hair cells could be beneficial for representing phasic receptor potentials with speed and fidelity [51, 81]. Differences in the properties of exocytosis between vestibular hair cells located in different regions of the vestibular organs (*e.g.*, striola/central compared to extrastriola/peripheral), remains to be determined.

While postsynaptic recordings have been made from the calyces of type-I hair cells [21, 23], no such data is available from the bouton terminals on type-II cells. However, a comparison of EPSCs recorded from vestibular calyces and the boutons innervating mature cochlear IHCs [92] reveals differences that reflect the presynaptic findings described above. The overall size of individual EPSCs is much smaller in calyces compared to IHC boutons, and there is an increase in EPSC amplitude with type-I hair cell depolarization that is not seen at cochlear boutons. Calyceal EPSCs have an unusually wide range of decays time constants, with some being very slow, presumably reflecting a substantial glutamate accumulation and spill over in the calyceal synaptic cleft [23]. The accumulation and spill over of glutamate within the very restricted volume of the synaptic cleft would provide a high synaptic gain to maximise the effect of the relatively small amount of glutamate release on the calyceal terminal. A more restricted release of glutamate from type-I hair cells could also prevent glutamate from rising to cytotoxic levels within the synaptic cleft during prolonged stimulation.

This could be a reason why a large, and potentially damaging, SRP in type-I hair cells may have been replaced by an alternative non-quantal mechanism of signal transmission [25].

Evidence for a non-quantal mode of transmission in type-I hair cells comes from the fact that neither *$Ca_V1.3$* nor *otoferlin* knockout mouse models show any serious vestibular deficit [71, 157]. Moreover, robust action potential activity could be elicited by hair cell depolarization in calyces from mature *$Ca_V1.3$* or *otoferlin* knockout mice, which was not affected by the AMPA receptor antagonist NBQX [153]. It is, therefore, possible that the lack of glutamate exocytosis in these transgenic mice is compensated by non-quantal, $Ca^{2+}$ independent signal transmission which has been shown to occur between type-I hair cells and their surrounding calyx terminal ([137, 158–161]; recently reviewed in [148, 149]). However, the capacity of non-quantal transmission to sustain vestibular function in the absence of chemical neurotransmission, requires further detailed investigation.

While the exact mechanism of non-quantal transmission remains unknown, it appears to involve voltage-gated $K^+$ channels expressed in the pre- and post-synaptic membranes facing the synaptic cleft [146, 162]. Different modes of action have been hypothesised, although they are not mutually exclusive: 1) $K^+$ exit from the basolateral membrane of the type-I hair cells directly depolarizes the calyx by changing the Nernst equilibrium potential across the inner calyx membrane [146, 155, 158, 159, 162]; 2) pre- and post-synaptic $K^+$ channels create a resistive coupling which allows for a direct (electrical) depolarization of the calyx [159] – note that electrical transmission typically involves gap junctions that are not present in the type-I hair cell-calyx synapse [125, 137]; 3) an ephaptic mechanism, whereby electrical fields created across narrowing and invagination of the synaptic space [125], can near-instantaneously influence the electrical excitability of the calyx [163], although there is currently no experimental support for this hypothesis.

The reasons why type-I vestibular hair cells require a dual, quantal and non-quantal, mode of signal transmission, are not clear. A restricted RRP of synaptic vesicles [153] might provide a transient (phasic) pulse of depolarization to the calyx, presumably to emphasise the transient component of the stimulus, as observed in calyceal afferents [123]. The potentially slower but sustained non-quantal transmission due to the accumulation of $K^+$ or glutamate in the synaptic cleft following hair cell depolarisation [23, 146] would provide a graded and tonic component of transmission or a sustained baseline level of calyx stimulation, which amplifies the effect of quantal transmission. Alternatively or additionally, if non-quantal transmission is a resistive or ephaptic coupling, it would provide a very fast (sub-millisecond) signal component [25, 137, 159, 164]. Such rapid transmission might be required for signalling jerk (the onset of acceleration: [164]) and for driving the rapid vestibulo-ocular reflex (VOR). The VOR, which stabilises gaze by counter-rotating the eyes during head rotation, is the fastest known reflex with a total latency as small as ~5 ms in the rhesus monkey [165].

While the exact functional roles of type-I and type-II vestibular hair cells remain to be determined, their very different biophysics and mechanisms of signal transmission across their synapses suggest they encode very different components of the vestibular input. The slower membrane kinetics and fully quantal mode of transmission onto multiple afferent contacts makes type-II cells suited for the slower, graded representation of head position. The rapid membrane kinetics and presence of a dual mode of, potentially instantaneous, transmission in type-I cells could specialise them for driving the rapid vestibular reflexes.

## 2.3 Hair cells loss and strategies for restoring auditory function

The loss of hair cells can result from several environmental insults including infectious agents, drugs such as aminoglycoside antibiotics or chemotherapeutics, trauma, loud sounds, a host of genetic factors, or ageing. Since mammalian hair cells do not regenerate, in contrast to those in birds and lower vertebrates (reviewed in [166]), their death produces permanent auditory/vestibular deficit [167, 168]. To date, most studies aimed at regenerating hair cells have focused upon cochlear hair cells. One reason for this is that loss of vestibular hair cells can be, at least partially, compensated by the other senses of vision and proprioception. There are currently three major strategies to restore hair cell function: stem-cell therapy, gene therapy, and molecular therapy (see [169–171]; for recent reviews). There has been an enormous advance in these methods in recent years, that we briefly discuss in the following sections. We refer to some excellent recent reviews on the different areas for those interested in exploring this topic further.

### *2.3.1 Stem-cell therapies*

Stem cells have the potential to self-renew and the ability to differentiate into multiple cell types. It is now well understood that a specific population of resident supporting cells, marked with the stem cell markers Lgr5, Lgr6, Sox2, Sox9, Frizzled-9, EPCAM, and ABCG2 in the organ of Corti, commonly known as cochlear stem/progenitor cells, hold the potential to proliferate and differentiate to form both hair cells and supporting cells (reviewed in [172]). There are two potential stem cell-based approaches that could be used to treat deafness. The first is the stimulation of the resident stem/progenitor cells within the inner ear to proliferate, therefore allowing them to replace the lost hair cells (reviewed in [173]). The basic limitation of this approach is the insufficient number of resident stem/progenitor cells in the inner ear, which means that only a small number of hair cells could potentially be replaced. The second approach is the exogenous supply of stem cells (stem cell transplantation) into the inner ear. The inner ear stem/progenitor cells, also called sensory precursor cells [174], are induced to re-enter the cell cycle by activating the inner ear-related signal pathways. Precursor cell proliferation and differentiation is normally regulated by various signalling pathways, including WNT, Notch, BMP/Smad, FGF, IGF, and Shh pathways [175–178]. The regulation of these pathways is very important for the induction of inner ear precursor cell differentiation into mature hair cells. In a recent review, Waqas et al. [172] discuss the potential for stem cells to combat sensorineural hearing loss in mammals, and explain their current therapeutic applications.

### *2.3.2 Gene therapies*

About fifty percent of inner ear disorders are caused by genetic mutations. Gene therapy is the treatment of diseases using genetic material (DNAs or RNAs). Recent progress in developing gene therapy treatments for genetic hearing loss has demonstrated tantalising proof-of-principle in animal models (see *e.g.*, [179, 180]). In their reviews, Ahmed et al. [181] and Shibata et al., [182] discuss progress, prospects, and challenges for gene therapy in the inner ear. They focus on technical aspects, including routes of gene delivery to the inner ear, choice of vectors, promoters, inner ear targets, therapeutic strategies, preliminary success stories, and points to consider for translating of these successes to the clinic.

### *2.3.3 Molecular therapies*

Pharmacological compounds that could induce generation of new hair cells would be particularly attractive for treating patients with hearing loss caused by hair cell death. A few studies have now reported generation of new hair cells by manipulating endogenous signalling pathways in supporting cells and in hair cells (reviewed in [171, 183]). In short, the differentiation efficiency of inner ear stem/progenitor cells into hair cells remains low. An insufficient number of new hair cells, immature new hair cells without the function of mature hair cells, and long–term survival of new hair cells are all key problems and difficulties that need to be resolved.

## 3. Conclusions

Mammalian hair cells are exquisite sensory receptors that, over hundreds of millions of years of evolution, have refined strategies to signal sound vibrations or head movements with amazing sensitivity and precision. Despite the notable increase in our knowledge of the mechanisms and molecules involved in hair cell function, there are still several aspects that remain to be elucidated. These include the intimate mechanisms controlling phase-locked quantal release in cochlear IHCs, and the nature of non-quantal transmission in vestibular type-I hair cells, as outlined above. Unfortunately, the complex molecular machinery responsible for sensory transduction and signalling make hair cells highly susceptible to several environmental ototoxic agents, genetic mutation, and ageing. Hair cells loss in mammals is permanent. Although the potential for regenerating hair cells appears to be present in adult mammalian inner ears, it requires their correct integration into an existing, mature organ. The new hair cells will need to be properly positioned in the epithelium according to their specific function (e.g., the hair cell type and location within the sensory epithelium), and properly innervated (*e.g.*, calyx or bouton terminal in the vestibular epithelia). However, precise localization and connectivity may not be an absolute requirement to restore significant functionality. As successful cochlear implants have demonstrated, the central auditory pathways are capable of learning how to interpret an imperfect or incomplete sensory input. The recent explosion of work using gene therapy to restore inner ear function in mouse models represents huge potential for the development future clinical applications for curing auditory and vestibular disorders in humans.

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## Conflict of interest

The authors declare no conflict of interest.

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