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Synaptic Studies Inform the Functional Diversity of Cochlear Afferents

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Abstract

Type I and type II cochlear afferents differ markedly in number, morphology and innervation pattern. The predominant type I afferents transmit the elemental features of acoustic information to the central nervous system. Excitation of these large diameter myelinated neurons occurs at a single ribbon synapse of a single inner hair cell. This solitary transmission point depends on efficient vesicular release that can produce large, rapid, suprathreshold excitatory postsynaptic potentials. In contrast, the many fewer, thinner, unmyelinated type II afferents cross the tunnel of Corti, turning basally for hundreds of microns to form contacts with ten or more outer hair cells. Although each type II afferent is postsynaptic to many outer hair cells, transmission from each occurs by the infrequent release of single vesicles, producing receptor potentials of only a few millivolts. Analysis of membrane properties and the site of spike initiation suggest that the type II afferent could be activated only if all its presynaptic outer hair cells were maximally stimulated. Thus, the details of synaptic transfer inform the functional distinctions between type I and type II afferents. High efficiency transmission across the inner hair cell's ribbon synapse supports detailed analyses of the acoustic world. The much sparser transfer from outer hair cells to type II afferents implies that these could respond only to the loudest, sustained sounds, consistent with previous reports from *in vivo* recordings. However, type II afferents could be excited additionally by ATP released during acoustic stress of cochlear tissues.

Introduction

Vertebrate sensory hair cells are canonical 'short receptors' having neither axons nor action potentials, but relying instead on transmitter release driven directly by the receptor potential that alters voltage-gated calcium influx. As in retinal photoreceptors and bipolar cells, transmitter release from hair cells occurs at ribbon synapses where vesicles are tethered to a dense body, and where voltage-gated calcium channels cluster in the plasma membrane (Nouvian et al., 2006; Matthews and Fuchs, 2010). Thus, ribbon synapse function will

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strongly influence information transfer from the inner ear to the CNS – particularly in the case of type I cochlear afferents whose single dendrite receives synaptic input from a single ribbon in one inner hair cell. This unusually solitary connection becomes still more intriguing given that activity patterns are thought to differ among the group of type I afferents contacting a single inner hair cell (Merchan-Perez and Liberman, 1996). Beyond the inner hair cell to type I afferent synapse however, still greater anatomical diversity is found in the cochlea (type II afferents) and vestibular end-organs (calyx, bouton, dimorphic afferents) whose functional diversity is only beginning to be revealed. Given the daunting complexity of vestibular synapses, this presentation will focus on a comparison of the structure and function of type I and type II cochlear afferents, with particular attention to details of transmitter release from their respective presynaptic hair cells.

Type I and type II afferents have distinctly different peripheral arbors (Fig. 1). The predominant type I afferents (90–95% of the total) are larger diameter, myelinated and extend a single unbranched dendrite to the inner hair cell row. The minority type II afferents are thinner and unmyelinated, with a peripheral process that crosses the tunnel of Corti then turns to extend hundreds of microns toward the cochlear base (Berglund and Ryugo, 1987). Each type II afferent gives off short branches to multiple outer hair cells, usually within one row (Brown, 1987; Jagger and Housley, 2003).

Type I afferents transmit fundamental aspects of acoustic stimuli including timing, intensity and frequency composition (Kiang et al., 1967; Sachs and Abbas, 1974; Sachs and Young, 1979; Young and Sachs, 1979; Young, 2008). Afferent frequency selectivity results from the specific innervation of single inner hair cells along the tonotopic axis of the cochlea; while the coding of timing and intensity requires transformation from the acoustic receptor potential to a related pattern of transmitter release onto the afferent dendrite (Fuchs, 2005; Moser et al., 2006). Multi-vesicular release from inner hair cell ribbon synapses may facilitate this transformation (Glowatzki and Fuchs, 2002; Wittig and Parsons, 2008; Goutman, 2012; Rutherford et al., 2012). The small caliber and scarcity of type II afferents severely limits single-unit recording, with only a small number reported, all insensitive to sound (Robertson, 1984; Brown, 1994; Robertson et al., 1999). Somatic recordings from excised tissue, or dissociated ganglion neurons have revealed some features of excitability (Jagger and Housley, 2003; Reid et al., 2004). More recent intracellular recordings from the spiral process have measured excitability and synaptic inputs, and support the notion that type II afferents could be activated only by the loudest sounds (Weisz et al., 2009; Weisz et al., 2012; Weisz et al., 2014).

Capacitance measurements from hair cells and postsynaptic recordings from afferent fibers have been used to document transmitter release from both inner and outer hair cells. Substantially more information exists for the inner hair cell to type I afferent synapse, using capacitance recording to detail developmental changes (Beutner and Moser, 2001; Johnson et al., 2009) and differences along the tonotopic axis (Johnson et al., 2008), as well as the influence of various genetic manipulations, e.g., knockout of bassoon (Khimich et al., 2005) or synaptotagmins (Beurg et al., 2010; Johnson et al., 2010; Reisinger et al., 2011). From such measurements it is possible to estimate the size of the readily-releasable pool (RRP) of vesicles (400–500 in gerbil IHCs (Johnson et al., 2009) corresponding to 10–14 vesicles per

ribbon; likewise 280 in mouse IHCs, corresponding to ~11 per each of 25 ribbons (Beutner and Moser, 2001). Immuno-label shows many fewer ribbons in most outer hair cells, consistent with their smaller total RRP (135–143 in gerbil OHCs – (Johnson et al., 2009)) and small voltage-gated calcium currents (Knirsch et al., 2007). However, the calcium current on a per ribbon basis seems to be about the same as for inner hair cells, as well as the number of vesicles in the RRP for each ribbon, 13 in OHCs of gerbil. Outer hair cells from newborn rats (P2–3) have many more ribbons, larger voltage-gated calcium currents and a far larger RRP = 1200, and these values all fall with maturation (Knirsch et al., 2007). These issues also have been examined by direct postsynaptic measurement of release probability – see below.

Type I afferents

Intracellular recording from type I afferent contacts on inner hair cells provides a privileged view of ribbon function with unmatched signal-to-noise and temporal resolution (Glowatzki and Fuchs, 2002). This vantage point has confirmed that glutamate released from the hair cell activates the AMPA class of postsynaptic receptors (Fig. 2A), and first revealed the wide (20-fold) range of amplitudes of stochastically-released postsynaptic currents (i.e., constant presynaptic membrane potential) (Fig. 2B). ‘Monophasic’ responses varied in amplitude but were characterized by uninterrupted rising and falling phases. Irregularly-shaped ‘multiphasic’ responses were generally smaller and seemingly composed of subunits. These and other observations suggested that the ribbon synapse can execute spontaneous multi-vesicular release, as suggested previously on the basis of sharp electrode recordings in the guinea pig cochlea (Siegel, 1992). How this might be accomplished remains uncertain, although the microstructure of the ribbon constrains calcium entering through nearby voltage-gated channels, creating locally-high clouds that could trigger release of already docked vesicles (Roberts, 1994; Graydon et al., 2011). The relatively large conductance of hair cell calcium channels (Zampini et al., 2014) could promote this process. Compound fusion of vesicles prior to release also has been proposed on the basis of ultrastructural studies (Matthews and Sterling, 2008). In the context of classical vesicular release hypotheses, multi-vesicular release would correspond to variations in quantum content (number of vesicles), whereas compound fusion would yield a variable quantal size. Variable quantal size also is the basis of a recent unquantal proposal (Chapochnikov et al., 2014). In this model, a chattering fusion pore releases variable amounts of glutamate from single fused vesicles. This in combination with an unsaturated pool of postsynaptic receptors can explain many features of release from inner hair cell ribbons, although perhaps not that from all hair cells. A wide range of postsynaptic current amplitudes also is found at hair cell afferent synapses in frog and turtle (Keen and Hudspeth, 2006; Li et al., 2009; Schnee et al., 2013). In both of these species, in contrast to the IHC afferent synapse, many ribbon synapses release onto a single afferent fiber. Current hypotheses regarding the underlying mechanisms include multi-vesicular release and ‘complex’ postsynaptic currents produced by temporal summation of overlapping release from multiple ribbons.

What all observations and models have in common is that the rate of transmitter release from hair cell ribbons is strongly calcium-dependent. This dependence takes the form of an increased rate of vesicular fusion events as cytoplasmic calcium increases. Paired pre- and

postsynaptic recordings established this fact for frog saccular hair cells (Keen and Hudspeth, 2006; Li et al., 2009) and at the ribbon synapse of inner hair cells (Goutman and Glowatzki, 2007). Progressive depolarization of mammalian inner hair cells increased the frequency but not mean amplitude of postsynaptic currents. This same conclusion was reached using different levels of cytoplasmic buffer in the hair cell to alter release frequency (Fig. 3). Paired recordings also provided a mechanism for adaptation of afferent activity. Postsynaptic currents declined to a lower steady-state during sustained depolarization of the presynaptic hair cell. This decline was shown to be due to decreased event frequency; presumably from depletion of the readily-releasable vesicular pool (the presynaptic calcium current was virtually constant). The rate of decline (initial time constant ~ 7 ms) was similar to that reported for adaptation of afferent firing rates to high frequency tone bursts (Taberner and Liberman, 2005), supporting the hypothesis that central signaling depends fundamentally on transmission at the ribbon synapse.

The main effect of presynaptic depolarization (calcium level in the hair cell) is to alter the rate of release events. This process is subject to depression (depletion) and voltage-dependent facilitation (Goutman and Glowatzki, 2011) that together set the release probability for any particular presynaptic membrane potential, as seen also for frog hair cell transmission (Cho et al., 2011). As a consequence, the peak timing of release remains constant at different release rates (Goutman, 2012), supporting the phase-constancy with intensity of afferent firing patterns seen for acoustic stimulation at the best frequency (Rose et al., 1967). A similar observation has been reported for transmitter release from hair cells of the frog amphibian papilla (Li et al., 2014).

The majority of these recordings were carried out on cochlear tissue excised from neonatal animals (before postnatal day 14), during a time when capacitance measurements indicate that release is immature (Beutner and Moser, 2001; Johnson et al., 2009). Postsynaptic recordings from older animals ($>P19$) revealed a shift toward larger, faster, monophasic events with relatively fewer multiphasic events (Grant et al., 2010). In older fibers the synaptic amplitudes could be normally distributed about a mean of several hundred pA (Fig. 4) – as though the mature pattern of release was for monophasic, multivesicular events, perhaps related to the observed higher efficiency coupling of calcium influx to capacitance increase (Beutner and Moser, 2001; Johnson et al., 2009). Nonetheless, some older synapses retained significant numbers of multiphasic events of lower amplitude, leading to the suggestion that these may serve low-spontaneous rate afferent fibers, with many more sub-threshold synaptic potentials.

Type II afferents

The same technique developed for type I afferents was adapted to make tight-seal, intracellular recordings from the peripheral processes of type II afferents as they spiral beneath the rows of outer hair cells in apical cochlear explants from 5–9 day-old rats (Weisz et al., 2009). These revealed typical, fast, ionotropic synaptic excitation by glutamate release from outer hair cells (Figure 5A). Sequential stimulation of individual outer hair cells showed that ten or more were presynaptic to a given type II afferent (Figure 5B). The strength of transmission from each hair cell was low, appearing to consist of single vesicles

released once for every four maximal presynaptic stimuli (Weisz et al., 2012). Similarly unquantal release seems to occur as well at the type I vestibular hair cell to calyx afferent synapse (Rennie and Streeter, 2006; Dulon et al., 2009; Eatock and Songer, 2011; Sadeghi et al., 2014).

Release was evoked onto type II afferents with a strong mechanical stimulus (waterjet puff) that reliably elicited calcium action potentials from these immature outer hair cells. Since this same stimulus was used in earlier studies on young inner hair cells (Beutner and Moser, 2001; Glowatzki and Fuchs, 2002), it is possible to compare the efficacy of transmission from young outer and inner hair cells stimulated to produce action potentials. One measure of synaptic strength is the quantum content, or average number of vesicles released for each presynaptic action potential, e.g., 200–300 at the mammalian neuromuscular junction (Boyd and Martin, 1956). Work on young inner hair cells suggested that each action potential caused the release of 40–50 vesicles at a single synaptic contact (Beutner et al., 2001; Glowatzki and Fuchs, 2002), in marked contrast to the very much lower probability of release from each outer hair cell onto type II afferents (0.25 vesicles per action potential). These comparisons inspire two types of questions: what might be the structural bases for these significant functional differences; and, what are the larger implications for cochlear signaling?

Many laboratories have described the presynaptic structures of outer hair cells, among them (Siegel and Brownell, 1981; Nadol, 1983; Simmons and Liberman, 1988; Hashimoto and Takasaka, 1989; Weisz et al., 2012). Immuno-labeled ribbons are relatively scarce, from 2–5 per outer hair cell, depending on species, development and cochlear position, see for example (Fujikawa et al., 2014) compared to the 20 or so found in the typical inner hair cell. Assuming that vesicular release from outer hair cells occurs at ribbons, as from inner hair cells, one might expect substantial differences in ribbon size, or the number and disposition of vesicles to account for the observed differences in transmitter release. However, a direct comparison in the apical turn of the young (P9) rat cochlea (equivalent to the locus of postsynaptic recordings) showed no difference in ribbon size and only a two to three-fold difference in vesicle numbers (Weisz et al., 2012). Outer hair cell ribbons were slightly taller and thinner (but with the same volume), with approximately half as many tethered and docked vesicles as found on inner hair cell ribbons. However, in addition to being fewer in number, vesicles at outer hair cell ribbons also appear to be more heterogeneous in size and disposition than those of inner hair cells (Fig. 6) as observed in studies of the developing and cultured mouse cochlea (Sobkowicz et al., 1982).

All together however, it is unclear how these relatively minor distinctions could explain the major differences in release efficiency between these types of hair cells. Both inner and outer hair cells can express otoferlin and synaptotagmin IV which are implicated in the linear calcium dependence of voltage-dependent capacitance measurements (Beurg et al., 2008; Dulon et al., 2009; Johnson et al., 2009; Johnson et al., 2010), but further characterization of component proteins may reveal differing molecular mechanisms. For example, the vesicular glutamate transporter VGlut3 appears to be absent from outer hair cells, but is required for transmission from inner hair cells (Akil et al., 2012). Another possibility is suggested by the observation that clusters of intra-membrane particles in freeze

fracture EM – thought to include voltage-gated calcium channels (Roberts et al., 1990)) are about 4-fold smaller under ribbons of outer hair cells compared to those of inner hair cells in the guinea pig cochlea (Saito, 1990). Clusters of postsynaptic intramembranous particles occur in type II afferents at a density of $3000/\mu\text{m}^2$ (Saito and Hama, 1984). While comparable numbers aren't available for the type I afferent, if these represent glutamate receptors one might expect larger such aggregations as another element of more effective transmission. The better-organized ribbons of most other hair cells are thought to ensure precise and reliable timing of release (Wittig and Parsons, 2008; Buran et al., 2010) to encode the information content of sound. By comparison, the outer hair cell to type II afferent connection seems poorly-designed for such coding.

Implications for function

Recognizing the limitations of room-temperature recordings from immature, excised cochlear tissue, nonetheless it is worth considering the implications of these results. Transmission from outer hair cells to type II afferents is extremely weak by comparison to that between inner hair cells and type I afferents in identical experimental conditions. Based on measurements of length constant using two intracellular electrodes, a compartmental model suggests that at least 6 outer hair cells (out of a pool of 20–25) would have to release a vesicle to bring the type II afferent to action potential threshold (Weisz et al., 2014). These estimates are based on maximal stimulation of the outer hair cell (calcium action potentials) – so presumably corresponding to maximal acoustic stimulation. If type I and II afferents project to similar regions of the cochlear nuclei (as HRP fills suggest - (Brown et al., 1988)) it is worth considering what combination of frequency and intensity might co-activate type II and I afferents. The synaptic input zone of type II fibers lies about $750\text{ }\mu\text{m}$ basal from the tunnel crossing (presumably where neighboring type I fibers reside). Thus, type II afferents will be co-activated by sounds loud enough for maximal basilar membrane vibration basal to the CF for a given type I afferent. This will occur as increasingly louder sounds result in asymmetric basal-ward extension of the traveling wave on the basilar membrane (Von Békésy, 1960).

Beyond acoustic stimulation, type II afferents respond vigorously to ATP (Fig. 7). It has been shown that damage to hair cells triggers a calcium-driven release of ATP among supporting cells (Gale et al., 2004), providing a potential source of type II excitation during acoustic trauma. Thus, type II afferents may be analogous to somatic C-fibers, i.e., nociceptors. Both are smaller diameter, unmyelinated, with wide-spread peripheral arbors that are sensitive to ATP. It has been shown that type II central terminals form synaptic contacts in the small cell cap and/or granule cell domain of the cochlear nuclei (Brown et al., 1988; Berglund and Brown, 1994; Benson and Brown, 2004) but the onward connections remain to be determined. Are there connections to central pain pathways?

Summary

Type I and type II cochlear afferents differ in morphology, innervation pattern and synaptic physiology, implying significantly different roles in cochlear function. It is well-established that type I afferents encode the information content of sound, and that efficacious transmitter

release from inner hair cell ribbon synapse enables that information transfer. The function of type II afferents remains unresolved, but weak synaptic transmission from outer hair cells reinforces the conclusion that information coding must be limited. An alternative hypothesis is that type II afferents are analogous to nociceptive C-fibers in skin, being small diameter, unmyelinated sensors with very high thresholds. That analogy is further strengthened by the sensitivity of type II fibers to ATP, a recognized noxious stimulant (Burnstock, 2013) released by supporting cells upon hair cell damage (Gale et al., 2004).

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Bibliography

- Akil O, Seal RP, Burke K, Wang C, Alemi A, During M, Edwards RH, Lustig LR. Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. *Neuron*. 2012; 75:283–293. [PubMed: 22841313]
- Benson TE, Brown MC. Postsynaptic targets of type II auditory nerve fibers in the cochlear nucleus. *J Assoc Res Otolaryngol*. 2004; 5:111–125. [PubMed: 15357415]
- Berglund AM, Ryugo DK. Hair cell innervation by spiral ganglion neurons in the mouse. *The Journal of comparative neurology*. 1987; 255:560–570. [PubMed: 3819031]
- Berglund AM, Brown MC. Central trajectories of type II spiral ganglion cells from various cochlear regions in mice. *Hearing research*. 1994; 75:121–130. [PubMed: 8071139]
- Beurg M, Safieddine S, Roux I, Bouleau Y, Petit C, Dulon D. Calcium- and otoferlin-dependent exocytosis by immature outer hair cells. *J Neurosci*. 2008; 28:1798–1803. [PubMed: 18287496]
- Beurg M, Michalski N, Safieddine S, Bouleau Y, Schneggenburger R, Chapman ER, Petit C, Dulon D. Control of exocytosis by synaptotagmins and otoferlin in auditory hair cells. *J Neurosci*. 2010; 30:13281–13290. [PubMed: 20926654]
- Beutner D, Moser T. The presynaptic function of mouse cochlear inner hair cells during development of hearing. *J Neurosci*. 2001; 21:4593–4599. [PubMed: 11425887]
- Beutner D, Voets T, Neher E, Moser T. Calcium dependence of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse. *Neuron*. 2001; 29:681–690. [PubMed: 11301027]
- Boyd IA, Martin AR. The end-plate potential in mammalian muscle. *The Journal of physiology*. 1956; 132:74–91. [PubMed: 13320373]
- Brown MC. Morphology of labeled afferent fibers in the guinea pig cochlea. *The Journal of comparative neurology*. 1987; 260:591–604. [PubMed: 3611412]
- Brown MC. Antidromic responses of single units from the spiral ganglion. *Journal of neurophysiology*. 1994; 71:1835–1847. [PubMed: 8064351]
- Brown MC, Berglund AM, Kiang NY, Ryugo DK. Central trajectories of type II spiral ganglion neurons. *The Journal of comparative neurology*. 1988; 278:581–590. [PubMed: 3230171]
- Buran BN, Strenzke N, Neef A, Gundelfinger ED, Moser T, Liberman MC. Onset coding is degraded in auditory nerve fibers from mutant mice lacking synaptic ribbons. *J Neurosci*. 2010; 30:7587–7597. [PubMed: 20519533]
- Burnstock G. Purinergic mechanisms and pain--an update. *European journal of pharmacology*. 2013; 716:24–40. [PubMed: 23524093]
- Chapochnikov NM, Takago H, Huang CH, Pangrsic T, Khimich D, Neef J, Auge E, Gottfert F, Hell SW, Wichmann C, Wolf F, Moser T. Uniquantal release through a dynamic fusion pore is a candidate mechanism of hair cell exocytosis. *Neuron*. 2014; 83:1389–1403. [PubMed: 25199706]

- Choi S, Li GL, von Gersdorff H. Recovery from short-term depression and facilitation is ultrafast and Ca^{2+} dependent at auditory hair cell synapses. *J Neurosci.* 2011; 31:5682–5692. [PubMed: 21490209]
- Dulon D, Safieddine S, Jones SM, Petit C. Otoferlin is critical for a highly sensitive and linear calcium-dependent exocytosis at vestibular hair cell ribbon synapses. *J Neurosci.* 2009; 29:10474–10487. [PubMed: 19710301]
- Eaton RA, Songer JE. Vestibular hair cells and afferents: two channels for head motion signals. *Annual review of neuroscience.* 2011; 34:501–534.
- Fuchs PA. Time and intensity coding at the hair cell's ribbon synapse. *The Journal of physiology.* 2005; 566:7–12. [PubMed: 15845587]
- Fujikawa T, Petralia RS, Fitzgerald TS, Wang YX, Millis B, Morgado-Diaz JA, Kitamura K, Kachar B. Localization of kainate receptors in inner and outer hair cell synapses. *Hearing research.* 2014; 314:20–32. [PubMed: 24858010]
- Gale JE, Piazza V, Ciubotaru CD, Mammano F. A mechanism for sensing noise damage in the inner ear. *Curr Biol.* 2004; 14:526–529. [PubMed: 15043820]
- Glowatzki E, Fuchs PA. Transmitter release at the hair cell ribbon synapse. *Nature neuroscience.* 2002; 5:147–154. [PubMed: 11802170]
- Goutman JD. Transmitter release from cochlear hair cells is phase locked to cyclic stimuli of different intensities and frequencies. *J Neurosci.* 2012; 32:17025–17035a. [PubMed: 23175853]
- Goutman JD, Glowatzki E. Time course and calcium dependence of transmitter release at a single ribbon synapse. *Proceedings of the National Academy of Sciences of the United States of America.* 2007; 104:16341–16346. [PubMed: 17911259]
- Goutman JD, Glowatzki E. Short-term facilitation modulates size and timing of the synaptic response at the inner hair cell ribbon synapse. *J Neurosci.* 2011; 31:7974–7981. [PubMed: 21632919]
- Grant L, Yi E, Glowatzki E. Two modes of release shape the postsynaptic response at the inner hair cell ribbon synapse. *J Neurosci.* 2010; 30:4210–4220. [PubMed: 20335456]
- Graydon CW, Choi S, Li GL, Kachar B, von Gersdorff H. Sharp Ca^{2+} nanodomains beneath the ribbon promote highly synchronous multivesicular release at hair cell synapses. *J Neurosci.* 2011; 31:16637–16650. [PubMed: 22090491]
- Hashimoto S, Takasaka T. Computer-aided serial section reconstruction of nerve endings on the outer hair cells of the cochlea. Semithin sections vs. ultrathin sections. *Acta oto-laryngologica.* 1989; 107:387–391. [PubMed: 2756829]
- Jagger DJ, Housley GD. Membrane properties of type II spiral ganglion neurones identified in a neonatal rat cochlear slice. *The Journal of physiology.* 2003; 552:525–533. [PubMed: 14561834]
- Johnson SL, Franz C, Knipper M, Marcotti W. Functional maturation of the exocytotic machinery at gerbil hair cell ribbon synapses. *The Journal of physiology.* 2009; 587:1715–1726. [PubMed: 19237422]
- Johnson SL, Forge A, Knipper M, Munkner S, Marcotti W. Tonotopic variation in the calcium dependence of neurotransmitter release and vesicle pool replenishment at mammalian auditory ribbon synapses. *J Neurosci.* 2008; 28:7670–7678. [PubMed: 18650343]
- Johnson SL, Franz C, Kuhn S, Furness DN, Rüttiger L, Munkner S, Rivolta MN, Seward EP, Herschman HR, Engel J, Knipper M, Marcotti W. Synaptotagmin IV determines the linear Ca^{2+} dependence of vesicle fusion at auditory ribbon synapses. *Nature neuroscience.* 2010; 13:45–52. [PubMed: 20010821]
- Keen EC, Hudspeth AJ. Transfer characteristics of the hair cell's afferent synapse. *Proceedings of the National Academy of Sciences of the United States of America.* 2006; 103:5537–5542. [PubMed: 16567618]
- Khimich D, Nouvian R, Pujol R, Tom Dieck S, Egner A, Gundelfinger ED, Moser T. Hair cell synaptic ribbons are essential for synchronous auditory signalling. *Nature.* 2005; 434:889–894. [PubMed: 15829963]
- Kiang NY, Sachs MB, Peake WT. Shapes of tuning curves for single auditory-nerve fibers. *The Journal of the Acoustical Society of America.* 1967; 42:1341–1342. [PubMed: 6081601]

- Knirsch M, Brandt N, Braig C, Kuhn S, Hirt B, Munkner S, Knipper M, Engel J. Persistence of Ca(v)1.3 Ca²⁺ channels in mature outer hair cells supports outer hair cell afferent signaling. *J Neurosci*. 2007; 27:6442–6451. [PubMed: 17567805]
- Li GL, Cho S, von Gersdorff H. Phase-locking precision is enhanced by multiquantal release at an auditory hair cell ribbon synapse. *Neuron*. 2014; 83:1404–1417. [PubMed: 25199707]
- Li GL, Keen E, Andor-Ardo D, Hudspeth AJ, von Gersdorff H. The unitary event underlying multiquantal EPSCs at a hair cell's ribbon synapse. *J Neurosci*. 2009; 29:7558–7568. [PubMed: 19515924]
- Matthews G, Sterling P. Evidence that vesicles undergo compound fusion on the synaptic ribbon. *J Neurosci*. 2008; 28:5403–5411. [PubMed: 18495874]
- Matthews G, Fuchs P. The diverse roles of ribbon synapses in sensory neurotransmission. *Nature reviews Neuroscience*. 2010; 11:812–822. [PubMed: 21045860]
- Merchan-Perez A, Liberman MC. Ultrastructural differences among afferent synapses on cochlear hair cells: correlations with spontaneous discharge rate. *The Journal of comparative neurology*. 1996; 371:208–221. [PubMed: 8835727]
- Moser T, Neef A, Khimich D. Mechanisms underlying the temporal precision of sound coding at the inner hair cell ribbon synapse. *The Journal of physiology*. 2006; 576:55–62. [PubMed: 16901948]
- Nadol JB Jr. Serial section reconstruction of the neural poles of hair cells in the human organ of Corti. II. outer hair cells. *Laryngoscope*. 1983; 93:780–791. [PubMed: 6855401]
- Nouvian R, Beutner D, Parsons TD, Moser T. Structure and function of the hair cell ribbon synapse. *The Journal of membrane biology*. 2006; 209:153–165. [PubMed: 16773499]
- Reid MA, Flores-Otero J, Davis RL. Firing patterns of type II spiral ganglion neurons in vitro. *J Neurosci*. 2004; 24:733–742. [PubMed: 14736859]
- Reisinger E, Bresee C, Neef J, Nair R, Reuter K, Bulankina A, Nouvian R, Koch M, Buckers J, Kastrop L, Roux I, Petit C, Hell SW, Brose N, Rhee JS, Kugler S, Brigande JV, Moser T. Probing the functional equivalence of otoferlin and synaptotagmin 1 in exocytosis. *J Neurosci*. 2011; 31:4886–4895. [PubMed: 21451027]
- Rennie KJ, Streeter MA. Voltage-dependent currents in isolated vestibular afferent calyx terminals. *Journal of neurophysiology*. 2006; 95:26–32. [PubMed: 16162827]
- Roberts WM. Localization of calcium signals by a mobile calcium buffer in frog saccular hair cells. *J Neurosci*. 1994; 14:3246–3262. [PubMed: 8182469]
- Roberts WM, Jacobs RA, Hudspeth AJ. Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J Neurosci*. 1990; 10:3664–3684. [PubMed: 1700083]
- Robertson D. Horseradish peroxidase injection of physiologically characterized afferent and efferent neurones in the guinea pig spiral ganglion. *Hearing research*. 1984; 15:113–121. [PubMed: 6490538]
- Robertson D, Sellick PM, Patuzzi R. The continuing search for outer hair cell afferents in the guinea pig spiral ganglion. *Hearing research*. 1999; 136:151–158. [PubMed: 10511634]
- Rose JE, Brugge JF, Anderson DJ, Hind JE. Phase-locked response to low-frequency tones in single auditory nerve fibers of the squirrel monkey. *Journal of neurophysiology*. 1967; 30:769–793. [PubMed: 4962851]
- Rutherford MA, Chapochnikov NM, Moser T. Spike encoding of neurotransmitter release timing by spiral ganglion neurons of the cochlea. *J Neurosci*. 2012; 32:4773–4789. [PubMed: 22492033]
- Sachs MB, Abbas PJ. Rate versus level functions for auditory-nerve fibers in cats: tone-burst stimuli. *The Journal of the Acoustical Society of America*. 1974; 56:1835–1847. [PubMed: 4443483]
- Sachs MB, Young ED. Encoding of steady-state vowels in the auditory nerve: representation in terms of discharge rate. *The Journal of the Acoustical Society of America*. 1979; 66:470–479. [PubMed: 512208]
- Sadeghi SG, Pyott SJ, Yu Z, Glowatzki E. Glutamatergic signaling at the vestibular hair cell calyx synapse. *J Neurosci*. 2014; 34:14536–14550. [PubMed: 25355208]
- Saito K. Freeze-fracture organization of hair cell synapses in the sensory epithelium of guinea pig organ of Corti. *J Electron Microsc Tech*. 1990; 15:173–186. [PubMed: 2355268]

- Saito K, Hama K. A freeze-fracture study of afferent and efferent synapses of hair cells in the sensory epithelium of the organ of Corti in the guinea pig. *Cell and tissue research*. 1984; 238:437–446. [PubMed: 6525614]
- Schnee ME, Castellano-Munoz M, Ricci AJ. Response properties from turtle auditory hair cell afferent fibers suggest spike generation is driven by synchronized release both between and within synapses. *Journal of neurophysiology*. 2013; 110:204–220. [PubMed: 23596330]
- Siegel JH. Spontaneous synaptic potentials from afferent terminals in the guinea pig cochlea. *Hearing research*. 1992; 59:85–92. [PubMed: 1629051]
- Siegel JH, Brownell WE. Presynaptic bodies in outer hair cells of the chinchilla organ of Corti. *Brain research*. 1981; 220:188–193. [PubMed: 7272751]
- Simmons DD, Liberman MC. Afferent innervation of outer hair cells in adult cats: II. Electron microscopic analysis of fibers labeled with horseradish peroxidase. *The Journal of comparative neurology*. 1988; 270:145–154. [PubMed: 3372736]
- Sobkowicz HM, Rose JE, Scott GE, Slapnick SM. Ribbon synapses in the developing intact and cultured organ of Corti in the mouse. *J Neurosci*. 1982; 2:942–957. [PubMed: 7097321]
- Taberner AM, Liberman MC. Response properties of single auditory nerve fibers in the mouse. *Journal of neurophysiology*. 2005; 93:557–569. [PubMed: 15456804]
- Von Békésy, G. Experiments in hearing. New York: McGraw-Hill; 1960.
- Weisz C, Glowatzki E, Fuchs P. The postsynaptic function of type II cochlear afferents. *Nature*. 2009; 461:1126–1129. [PubMed: 19847265]
- Weisz CJ, Glowatzki E, Fuchs PA. Excitability of type II cochlear afferents. *J Neurosci*. 2014; 34:2365–2373. [PubMed: 24501375]
- Weisz CJ, Lehar M, Hiel H, Glowatzki E, Fuchs PA. Synaptic transfer from outer hair cells to type II afferent fibers in the rat cochlea. *J Neurosci*. 2012; 32:9528–9536. [PubMed: 22787038]
- Wittig JH Jr, Parsons TD. Synaptic ribbon enables temporal precision of hair cell afferent synapse by increasing the number of readily releasable vesicles: a modeling study. *Journal of neurophysiology*. 2008; 100:1724–1739. [PubMed: 18667546]
- Young ED. Neural representation of spectral and temporal information in speech. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2008; 363:923–945. [PubMed: 17827107]
- Young ED, Sachs MB. Representation of steady-state vowels in the temporal aspects of the discharge patterns of populations of auditory-nerve fibers. *The Journal of the Acoustical Society of America*. 1979; 66:1381–1403. [PubMed: 500976]
- Zampini V, Johnson SL, Franz C, Knipper M, Holley MC, Magistretti J, Russo G, Marcotti W, Masetto S. Fine Tuning of CaV1.3 Ca²⁺ channel properties in adult inner hair cells positioned in the most sensitive region of the Gerbil Cochlea. *PloS one*. 2014; 9:e113750. [PubMed: 25409445]

Highlights

- Hair cell to afferent synapses differ markedly in efficacy.
- Single inner hair cell ribbons activate each type I afferent.
- Multiple presynaptic outer hair cells only weakly excite each type II afferent.
- Type II afferents respond strongly to ATP, a noxious stimulant.

Figure 1.
Typical morphology of type I and type II afferents as shown by HRP fills in the mouse cochlea. (From Berglund and Ryugo, 1987, with permission).

Figure 2.

Stochastic postsynaptic currents in type I afferent (hair cell at rest or subject to potassium depolarization in excised apical turn of young rat cochlea). A. Rapidly gating inward currents were sensitive to cyclothiazide, a specific modulator of AMPA-type glutamate receptors. B. Spontaneous postsynaptic currents varied widely in amplitude within each recording. (Reproduced from Figures 2 and 3, Glowatzki and Fuchs, 2002, with permission)

Figure 3.

Pair recordings from inner hair cell and type I afferent dendritic bouton (young rat cochlea). Transmitter release was evoked under different conditions of calcium buffering. An increase of intracellular EGTA concentration, or change from EGTA to BAPTA greatly reduced the frequency, but not average size of evoked synaptic currents. (From Figure 4, Goutman and Glowatzki 2007, copyright © by the National Academy of Sciences)

Figure 4.

Postsynaptic currents in type I afferents of the mature rat cochlea. Cumulative fraction plots (A, D) and histograms (B, C, E, and F) illustrate the amplitude distribution for EPSCs in type I afferents. These tend to cluster around much larger mean amplitudes than in younger tissue. Note also that small events still occur in some recordings from mature cochlear tissue (from Grant et al., 2010, Figure 7, with permission).

Figure 5.

Synaptic inputs from outer hair cells (OHCs) to type II afferents. A. Examples of excitatory synaptic currents (EPSCs) evoked by waterjet stimulation of OHC. Waterjet pulse begins at time 0 and elicits regenerative depolarizations (calcium spikes) from OHCs whose half-amplitude duration extends from 7–40 ms (as determined in separate recordings). B. Mapping synaptically-coupled OHCs. Red circles are OHCs that produced EPSCs when stimulated, blue were silent. Black circles indicate position of type II recording pipette. Amplitude histograms of EPSCs from near (@ 30 μm) and far (@ 109 μm) OHCs had similar distributions and little amplitude variation, indicating single events (vesicles), and no cable loss over that distance (single exemplar – cell F - of amplitude histograms summarized in Figs. 6 and 7, Weisz et al., 2012).

Figure 6.
Ribbons and vesicles of inner hair cells (IHC) and outer hair cells (OHCs) from the apical turn of a P9 rat cochlea (Lehar, Hiel and Fuchs, unpublished).

Figure 7. ATP stimulates type II afferents. A. ATP triggers inward current in voltage-clamp, and B. large depolarization from the resting potential (from Weisz et al., 2009, Figure 3, with permission).