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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.

1. 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 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

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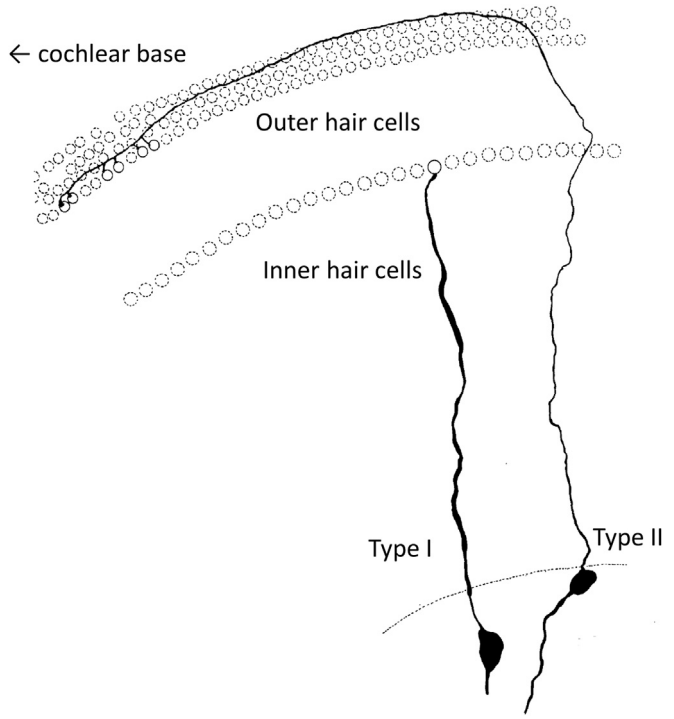

Fig. 1. Typical morphology of type I and type II afferents as shown by HRP fills in the mouse cochlea. (From Berglund and Ryugo, 1987, with permission).

(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, 2012, 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.

2. 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 uniquantal 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

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