



The spiral ganglion: Connecting the peripheral and central auditory systems

Bryony A. Nayagam^a, Michael A. Muniak^b, David K. Ryugo^{b,c,d,*}

^a Department of Otolaryngology, University of Melbourne, Melbourne, VIC, Australia

^b Department of Neuroscience, Johns Hopkins University, Baltimore, MD, USA

^c Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University, Baltimore, MD, USA

^d Garvan Institute, Darlinghurst, NSW, Australia

ARTICLE INFO

Article history:

Received 8 December 2010

Received in revised form

26 March 2011

Accepted 3 April 2011

Available online 21 April 2011

ABSTRACT

In mammals, the initial bridge between the physical world of sound and perception of that sound is established by neurons of the spiral ganglion. The cell bodies of these neurons give rise to peripheral processes that contact acoustic receptors in the organ of Corti, and the central processes collect together to form the auditory nerve that projects into the brain. In order to better understand hearing at this initial stage, we need to know the following about spiral ganglion neurons: (1) their cell biology including cytoplasmic, cytoskeletal, and membrane properties, (2) their peripheral and central connections including synaptic structure; (3) the nature of their neural signaling; and (4) their capacity for plasticity and rehabilitation. In this report, we will update the progress on these topics and indicate important issues still awaiting resolution.

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1. Spiral ganglion

In the inner ear, a channel formed by a latticework of bone spirals around in parallel to the coiled labyrinth. This channel is called Rosenthal's canal within which reside the somata of the spiral ganglion neurons. Each cell body emits a peripheral process that extends toward the organ of Corti and a central process that projects into the auditory nerve. Two populations of neurons have been described in the spiral ganglion (Fig. 1A). The classification has been made on the basis of somatic size, relative abundance, cytologic traits, and characteristics of the central and peripheral

processes (Munzer, 1931; Thomsen, 1966; Kellerhals et al., 1967; Spoendlin, 1971, 1973; Kiang et al., 1984).

Type I ganglion cells are large, bipolar, and represent 90–95% of the population. The cytoplasm is characterized by the plentiful ribosomes, cisternae of endoplasmic reticulum, and Golgi bodies. Other inclusions can also be observed including multivesicular, granular, and lipid bodies (Rosenbluth, 1962; Spoendlin, 1981). The cell body is encased in relatively loose myelin, the nucleus is pale and centrally located, and there is a distinct absence of cytoplasmic neurofilaments. The human type I ganglion cells are different because they are not usually myelinated (Kimura et al., 1976; Ota and Kimura, 1980). Their processes, however, are wrapped by 20–30 layers of compact myelin (Thomsen, 1966).

Type II ganglion cells are small, bipolar or pseudomonopolar, and represent 5–10% of the population. The cytoplasm is highly filamentous but otherwise lacking in the usual organelles (Spoendlin, 1973). The nucleus tends to appear darker than that of type I cells and may appear lobulated and eccentric in position. The myelin on these small cells is variable. In cats, for example, myelination of small cells is rare (Spoendlin, 1971), but approximately half the small cells in humans have a thin myelin sheath (Ota and Kimura, 1980).

2. Cytoskeletal properties of the spiral ganglion neurons

On the basis of cytoplasmic and cytoskeletal contents, the type I and II spiral ganglion neurons can be distinguished using different kinds of stains. Basic dyes stain nucleic acids (DNA and RNA),

Abbreviations: ab, ascending branch; AN, auditory nerve; ANN, auditory nerve nucleus; ANr, auditory nerve root; AVCN, anteroventral cochlear nucleus; BDNF, brain-derived neurotrophic factor; CF, characteristic frequency; CNTF, ciliary derived neurotrophic factor; db, descending branch; dB, decibel; SPL, sound pressure level; DCN, dorsal cochlear nucleus; DNA, deoxyribonucleic acid; EB, endbulb; GDNF, glial cell derived neurotrophic factor; HRP, horseradish peroxidase; IHCs, inner hair cells; kDa, kilodalton; kHz, kilohertz; LIF, leukemia inhibitory factor; mRNA, messenger ribonucleic acid; NFL, neurofilament light chain; NFM, neurofilament medium chain; NFH, neurofilament heavy chain; NGF, nerve growth factor; NT3, neurotrophin-3; NT4/5, neurotrophin-4/5; OHCs, outer hair cells; OSFs, outer spiral fibers; PSD, post-synaptic density; PVCN, posteroventral cochlear nucleus; RNA, ribonucleic acid; SR, spontaneous discharge rate; Th, threshold; Trk, tyrosine receptor kinase; TrkB, tyrosine receptor kinase-B; TrkC, tyrosine receptor kinase-C.

* Corresponding author. Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia. Tel.: +612 9295 8288.

E-mail address: d.ryugo@garvan.org.au (D.K. Ryugo).

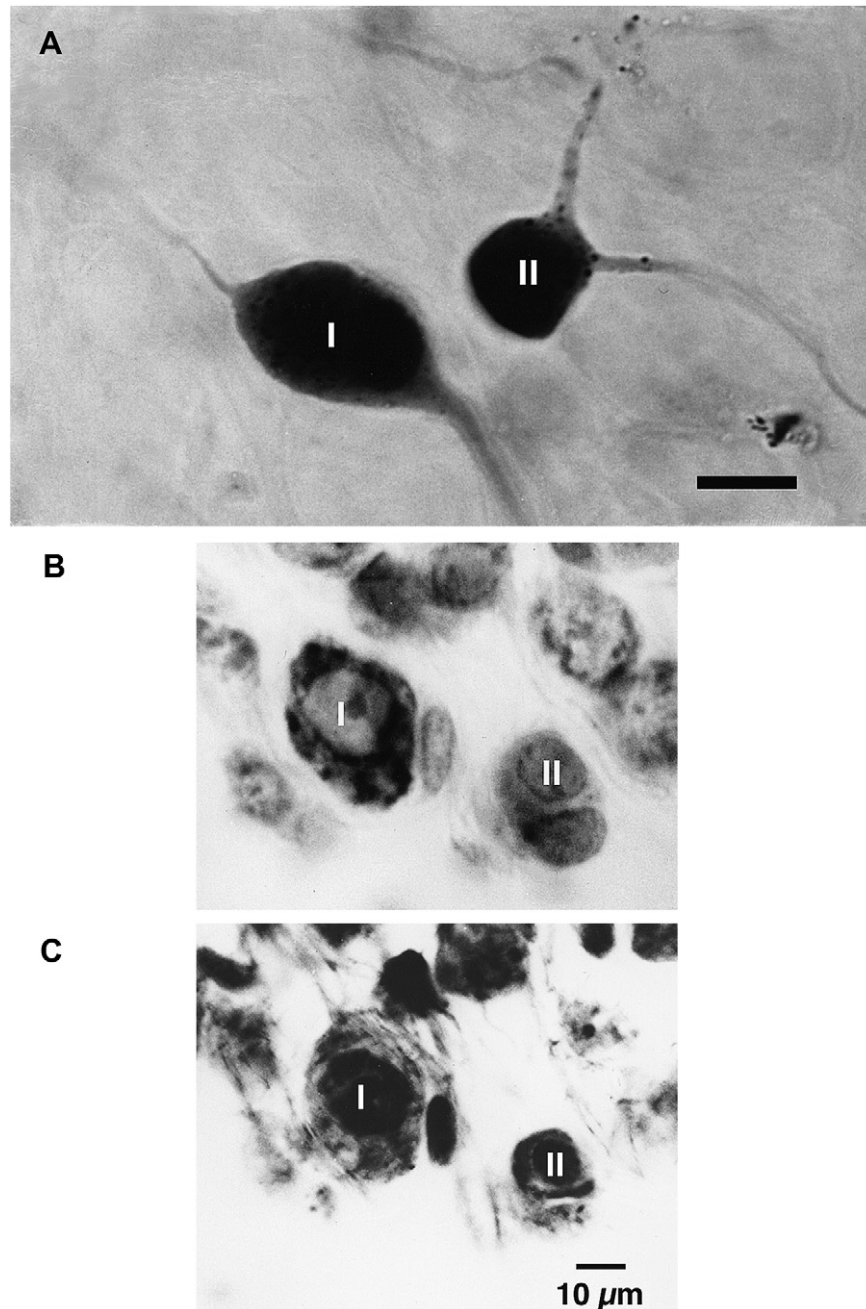


Fig. 1. Light micrographs of representative type I and type II spiral ganglion neurons. (A) HRP-labeled neurons from a cat that reveal the central and peripheral processes. Note for the bipolar type I neuron, the peripheral process (left side) is very thin ($<0.5 \mu\text{m}$), in comparison to the central process (right side). In contrast, the processes of the pseudo-monopolar type II neuron are approximately equal in caliber. Scale bar equals $10 \mu\text{m}$ (Kiang et al., 1982) (B) Cresyl violet stained type I and II neurons from a rat. Note that the cytoplasm of the type I neurons is prominent and blotchy with a pale nucleus; in contrast, the type II cytoplasm is pale. This staining illustrates the differential content of Nissl substance. (C) Protargol stained cells from panel B after cresyl violet was rinsed off. Note that the type I cytoplasm does not stain much for neurofilaments, whereas the cytoplasm of the type II neuron does. (From Berglund and Ryugo, 1986).

thereby imparting dye onto ribosomes and chromatin of all cells, including endothelial cells (that comprise blood vessels), smooth muscle cells (that encircle arterioles), Schwann cells that form myelin, and neurons. Although type I neurons will stain darker than the filamentous type II neurons, the differences are subtle (Fig. 1B). An alternative method is to stain for filaments so that type II neurons appear more conspicuous (Fig. 1C). Type II neurons have been stained using variations of silver stains (Berglund and Ryugo, 1986), antibodies directed against the phosphorylated 200 kDa neurofilament protein (Berglund and Ryugo, 1986, 1991), and

peripherin, a neuron-specific intermediate filament protein (Hafidi et al., 1993; Hafidi, 1998), described below. The obvious staining of type II neurons using these methods (Fig. 2) yields a nearly all-or-none reaction so that determining their uniform distribution throughout the length of the spiral ganglion was possible (Berglund and Ryugo, 1991).

The neurofilament proteins are expressed in both type I and II spiral ganglion neurons of the cochlea, where they play a key role in maintaining the structural integrity and normal growth of these neurons during both development and adulthood (Berglund and

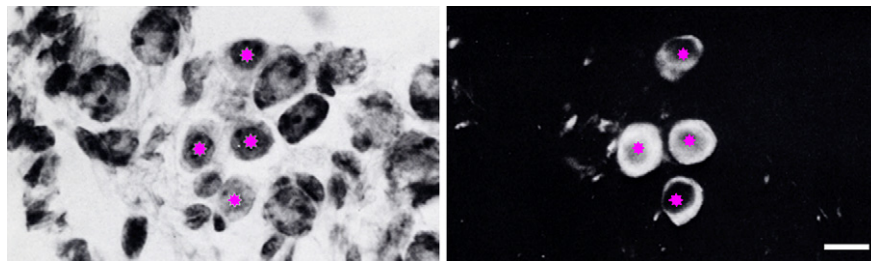


Fig. 2. Photomicrograph of spiral ganglion of rat stained using (A) cresyl violet and (B) fluorescent antibody directed against RT-97 that stains the phosphorylated 200 kD neurofilament protein. The light staining cytoplasm of type II neurons using cresyl violet is prominently stained by the antibody, RT-97. This selective and distinctive staining allowed the authors to determine that type II ganglion neurons were uniformly distributed along the length of Rosenthal's canal. Scale bar equals 10 μ m (From Berglund and Ryugo, 1986).

Ryugo, 1986, 1991; Romand et al., 1988, 1990; Dau and Wenthold, 1989; Hafidi et al., 1990; Kuijpers et al., 1991). Three interrelated subunits of neurofilament proteins are commonly referred to as the neurofilament protein triplet. The neurofilament triplet comprises proteins with characteristic molecular weights, light (68 kDa), medium (160 kDa) and heavy (200 kDa), upon which the nomenclature of the neurofilaments is derived (NFL, NFM and NFH, respectively: Hoffman and Lasek, 1975; Scott et al., 1985). In the developing nervous system, the neurofilament triplet proteins are sequentially expressed, with the light and medium molecular weight subunits appearing first in immature neurons, followed by the later expression of the heavy molecular weight subunit in more mature neurons (Shaw and Weber, 1982; Carden et al., 1987). A similar pattern of expression has been observed in the developing mammalian cochlea with the stage-specific expression of NFL and NFM prior to NFH (Hafidi et al., 1990; Tonnaer et al., 2010).

Immunoreactivity to NFL and NFM was observed in spiral ganglion neuron somata and their processes on embryonic day 16 in the developing rat cochlea, with NFH expression first detected at embryonic day 20 in the fibers of the spiral ganglion and at birth in the somata of the ganglion neurons (Hafidi et al., 1990). A more recent study comparing phosphorylation dependent and independent monoclonal antibodies for the neurofilament protein triplet detected the first expression of NFL in the rat spiral ganglion at embryonic day 12 (Tonnaer et al., 2010). This result is consistent with earlier studies (Carden et al., 1987), in which the first expression of NFL and NFM was identified at embryonic day 12 after early differentiation events in the developing rat brain. In addition, this study illustrated that non-phosphorylated NFH proteins could be partially detected in the spiral ganglion neurons from embryonic day 18, but phosphorylated NFH epitopes were not detected after birth (Tonnaer et al., 2010). It is noteworthy that the expression of the neurofilament proteins follows a basal to apical gradient (Tonnaer et al., 2010), which coincides with the general spatiotemporal pattern of differentiation, innervation and development in the developing mammalian cochlea (Pirvola et al., 1991; Rubel and Fritzsche, 2002).

The phosphorylation status of the NFH subunit has been illustrated to be an important factor in characterizing the two subtypes of spiral ganglion neurons in the cochlea. Using a monoclonal antibody against phosphorylated NFH (RT-97), type II spiral ganglion somata were shown to be preferentially labeled in the adult mouse, gerbil, rat, cat and human cochlea (Berglund and Ryugo, 1986, 1991). Importantly, when cochlear sections from these species were treated with alkaline phosphatase (to remove the phosphate groups), no staining was observed with the phosphorylated NFH antibody. Several other neurofilament antibodies did, however, label both spiral ganglion neuron subtypes in these cochlea. The cytoplasmic presence of phosphorylated NFH is both a rare and interesting observation, and has important implications in terms of function of the type II spiral ganglion neurons in the cochlea.

Phosphorylation of NFH has been proposed to be involved in fast axonal transport, resistance of neurofilament degradation, myelin formation, and development of axon caliber (Cole et al., 1994; Gotow et al., 1999; Shea et al., 2003, 2004). Why the somata of type II neurons contain the phosphorylated NFH remains to be determined. Given that the type II spiral ganglion neurons are unmyelinated, it is conceivable that they require additional phosphorylated NFH in order to stabilize the cytoskeleton, or perhaps their role requires high levels of phosphate to drive cellular processes.

The type II spiral ganglion neuron-specific expression of phosphorylated NFH in the somata presumably plays an important anatomical and functional role, although this idea has not yet been thoroughly explored. When NFH was over-expressed in a transgenic mouse strain, causing an increase in the somatic neurofilament content, the unexpected result was a dramatic increase in the lifespan of these otherwise unaffected animals (Couillard-Despres et al., 1998). In addition, when investigations were carried out to determine whether this effect was due to accumulation of neurofilaments in the somata as opposed to depletion of neurofilaments in the processes of the cell, it was reported that a 40% reduction in axonal neurofilaments did not alter the lifespan of the animals (Nguyen et al., 2000). Collectively, these findings suggest that the presence of neurofilament proteins in the cell body may confer protective benefits in neurodegenerative conditions. This conclusion may have important implications for spiral ganglion neuron survival after deafness, as it supports the observation that type II spiral ganglion neurons have a survival advantage following nerve damage.

The differential survival of small ganglion neurons after auditory nerve transection provided the first inkling that the two types of ganglion cells had separate innervation patterns (Spoendlin, 1971, 1973). Transection of the auditory nerve resulted in a loss of the large, myelinated cell bodies of spiral ganglion cells and afferent synaptic endings under the inner hair cells (IHCs). What remained were the small, unmyelinated somata of the spiral ganglion and afferent endings under outer hair cells (OHCs). These observations were used as a strong inference that the large, type I neurons innervated IHCs, whereas the small type II neurons innervated the OHCs. The appearance of some very large, multinucleated neurons that were not present in normal ganglia could not be explained (Spoendlin, 1975). Because this evidence was indirect (processes were never continuously traced from ending under hair cell to cell body) and derived under pathologic conditions, the final conclusion regarding peripheral innervation awaited the direct proof that was eventually provided (Kiang et al., 1982).

Although the idea about survival advantages is not supported by clinical data obtained from human temporal bones (Zimmermann et al., 1995; Nadol, 1997), the more rapid degeneration of spiral ganglion neurons observed in animal models of deafness, matched with the ability to use appropriate fixatives and controls, may give

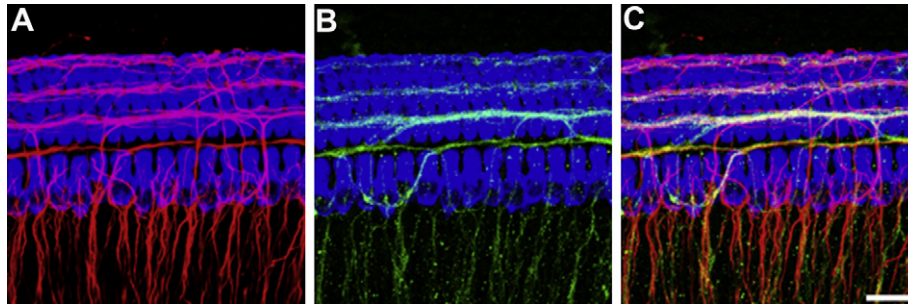


Fig. 3. Fluorescence confocal microscopy illustrating type II innervation of outer hair cells in the apical region of early post-natal rat cochlea. Using antibodies against peripherin and neurofilaments, type I processes (peripherin negative) can be readily distinguished from type II processes (peripherin positive), facilitating experimental studies of innervation, synaptogenesis and remodeling in the early post-natal cochlea. Myosin VIIa positive hair cells are shown in blue (A–C), type I and II ganglion neurons processes are shown in red (neurofilament 200 kDa; A) and type II specific peripherin expressing processes are shown in green (B). All three labels are overlaid in (C). Nayagam, unpublished data; scale bar equals 20 μm (A–C).

a better indication of whether type II spiral ganglion neurons are more resistant to degeneration following different onsets of deafness. The ongoing discoveries of the importance of the neurofilament proteins in degenerative diseases (Perrot et al., 2008) coupled with indications that type II ganglion neurons degenerate at a slower rate than type I neurons in the deafened rat cochlea (Bichler et al., 1983) suggest a possible avenue for future investigations.

Peripherin is a type III intermediate filament, which has been detected in a subset of sensory, motor and autonomic neurons, many of which extend their processes into the peripheral nervous system (Escurat et al., 1990). Although roles in axon growth, guidance and regeneration have been proposed, the precise function of peripherin remains contentious (Lariviere and Julien, 2004) and the mechanisms that regulate its expression in the cochlea are yet to be defined (Hafidi, 1998). Peripherin expression was first observed at embryonic day 16 in the developing rat cochlea, where it was detected in neuronal somata throughout the spiral ganglion and found to colocalize with NFM (Hafidi et al., 1993). After birth, peripherin immunoreactivity was observed to decrease in the spiral ganglion, and by adulthood, had become restricted to a subset of ganglion neurons. This subset of neurons was determined to be type II ganglion neurons, on the basis of their size, colocalization with NFM, and decreased peripherin labeling in the fibers beneath the IHCs, but not OHC innervation (Hafidi et al., 1993). The observation that peripherin expression becomes restricted to discrete classes of neurons during maturation is in line with related studies in the mammalian central nervous system (Escurat et al., 1990; Gorham et al., 1990).

Strong peripherin immunoreactivity is reported in both the somata and processes of the type II spiral ganglion neurons, similar to phosphorylated NFH (Hafidi et al., 1993; Hafidi, 1998). The selective expression of peripherin in the type II ganglion neurons of the adult mammalian cochlea is thought to contribute to stabilization of both the central and peripheral fiber projections of these cells (Hafidi, 1998). This hypothesis is logical given that type II neurons project longer distances in both directions compared to the type I neurons, by sending a central projection to the granule cell layer in the cochlear nucleus (Brown et al., 1988) and a peripheral projection to innervate numerous OHCs in a divergent manner (Kiang et al., 1982; Berglund and Ryugo, 1987; Simmons and Liberman, 1988). Moreover, this selective pattern of peripherin expression in type II spiral ganglion neurons (and dextran for type I ganglion neurons) has been used to help elucidate the mechanisms behind neurite outgrowth and synaptic remodeling in the developing mouse (Huang et al., 2007), and rat cochlea (Fig. 3).

There remain confounding differences when comparing data between *in vivo* and *in vitro* models. Whilst the type II peripheral

processes are clearly peripherin immunopositive in explants from the early post-natal rat cochlea (Fig. 3), the proportion of somata of spiral ganglion neurons showing peripherin expression is higher than might otherwise be expected to account for type II ganglion neurons alone (Fig. 4). This result may be explained by the previous observation that type II spiral ganglion cells tend to occupy the periphery of the spiral ganglion (Berglund and Ryugo, 1987). However, it also raises the following kinds of questions: are there relatively more type II neurons in the neonatal rat cochlea than in the mature cochlea? Can we expect a large amount of type II apoptosis during early development? Do type II neurons convert to type I neurons? How does peripherin immunostaining change with age and does it change further following deafness?

The data also highlight conflicts from *in vivo* studies where the results of electrical stimulation are reported to enhance ganglion cell survival or to have no effect (e.g., Hartshorn et al., 1991; Leake

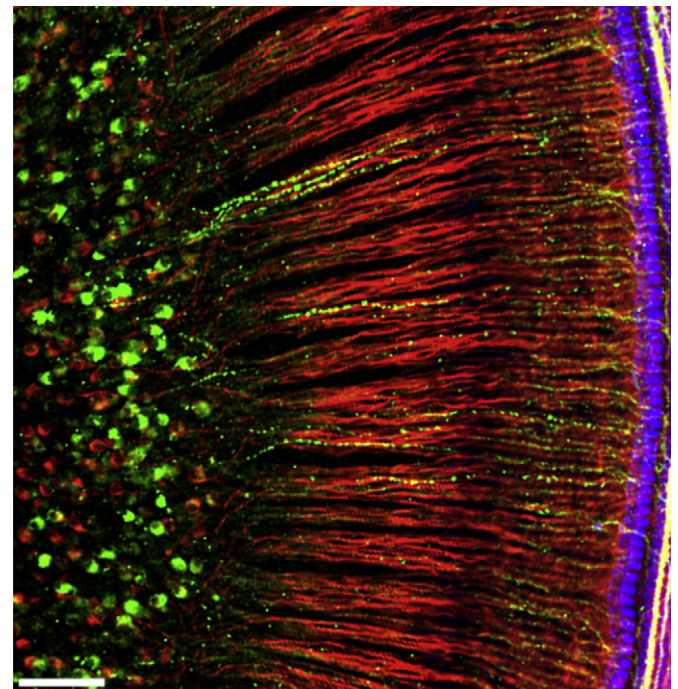


Fig. 4. Type I and II spiral ganglion neuron ratio and innervation pattern in the early post-natal rat cochlea, shown using type II specific peripherin (green), pan neuronal marker, neurofilament (red) and Myosin VIIa positive hair cells (blue). Low magnification confocal micrograph depicts large numbers of type II peripherin positive (green) somata located at the periphery of the spiral ganglion. Nayagam, unpublished data; scale bar equals 100 μm .

et al., 1991, 1999; Mitchell et al., 1997; Araki et al., 1998; Coco et al., 2007). Support for either conclusion using *in vitro* data (e.g., Hansen et al., 2001) may not necessarily be valid. *In vitro* experiments have provided powerful and valuable models for investigations, yet are only approximate indicators of what might happen in an intact system because they represent unnatural environments. Interpretation of results across experimental conditions must be made with caution, given the large (70%) and rapid (42 h) demise of ganglion cells in culture, coupled with striking differences in morphology (Whitlon et al., 2006), differences in electrophysiological properties (Needham et al., 2010; Mistrik et al., 2011), and changes in expression of synaptic proteins (Glowatzki, personal communication) compared to *in vivo* results. The challenge is to utilize these observations to derive testable hypotheses and to identify the limitations of each type of experimental approach to optimally further our understanding of type I and II spiral ganglion neurons and cochlear biology.

3. The peripheral process of type I and type II spiral ganglion neurons

3.1. Type I neurons

One of the striking and consistent features of the type I neuron across species is the thin caliber of its peripheral process (Figs. 1 and 5). This difference was mentioned in an ultrastructural study of the guinea pig spiral ganglion (Thomsen, 1966) and quantified as a ratio

of central process diameter to peripheral process diameter for a variety of mammals, including cat, mouse, opossum, guinea pig, squirrel monkey, and human (Kiang et al., 1982, 1984; Berglund and Ryugo, 1986). Interestingly, the thin axoplasmic segment of the peripheral process is completely myelinated with no apparent thinning of the sheath (Goycoolea et al., 1990). These authors speculated that the thin peripheral process could seal itself quickly in response to trauma, thereby facilitating survival of the cell body.

The peripheral processes, called radial fibers, emanate from the ganglion and project across the osseous spiral lamina, which is a bony shelf that extends from Rosenthal's canal and anchors the medial edge of the basilar membrane. The fibers cross the osseous spiral lamina in a radial direction without branching. In the osseous spiral lamina, the diameter of the myelinated fibers toward the scala vestibuli side is thinner compared to those on the scala tympani side (Kawase and Liberman, 1992). There are three internodal segments of peripheral myelin, approximately 150–200 μm in length, along this trajectory (Liberman and Oliver, 1984) before it passes through the habenula perforata (Spoendlin, 1973). The process is unmyelinated when it enters the cochlear duct and approaches the IHC.

Cochlear frequency maps have been derived for various mammals by labeling auditory nerve fibers that have been physiologically characterized. In the cat, individual auditory nerve fibers were intracellularly injected with horseradish peroxidase after determining their characteristic frequency (CF), that frequency to which they were most sensitive (Liberman, 1982). In the mouse, rat

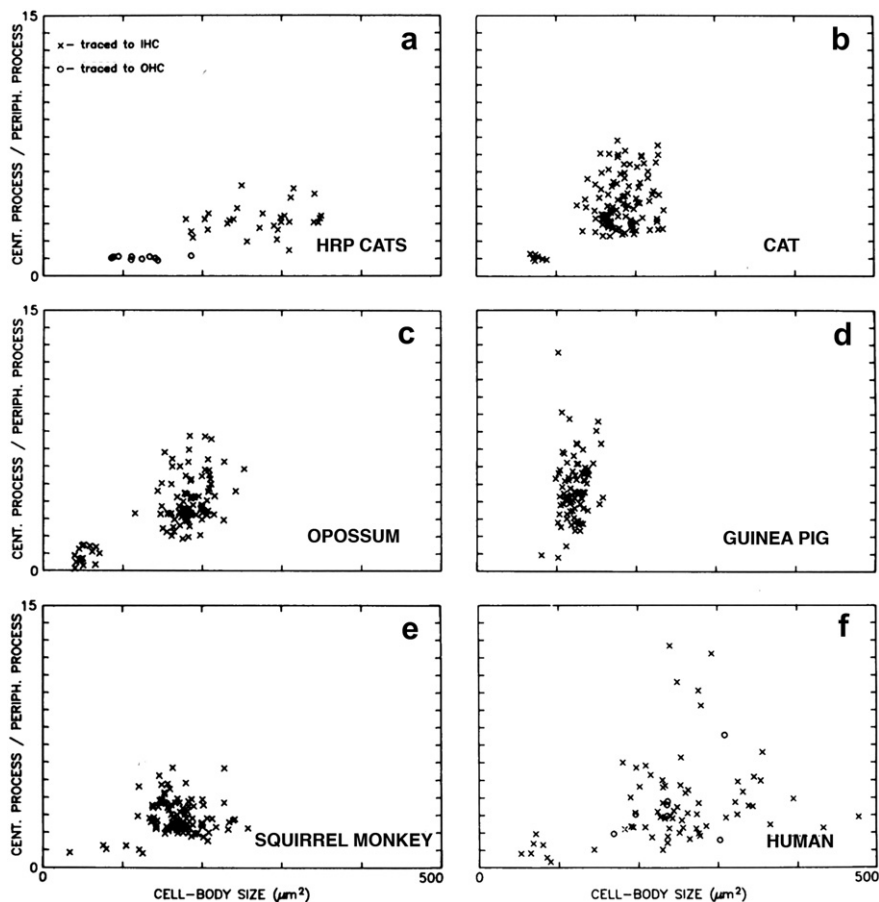


Fig. 5. These plots compare somatic cell sizes and process-size ratios for samples of spiral ganglion cells from a variety of species. Somatic size is represented by silhouette area. For the human data only (f), circles represent data from pseudomonopolar cells, collected from a 38-year-old male without a history of hearing loss who died from bladder cancer. Note how the two cell populations segregate into separate clusters on the basis of cell size and process ratio (From Kiang et al., 1984).

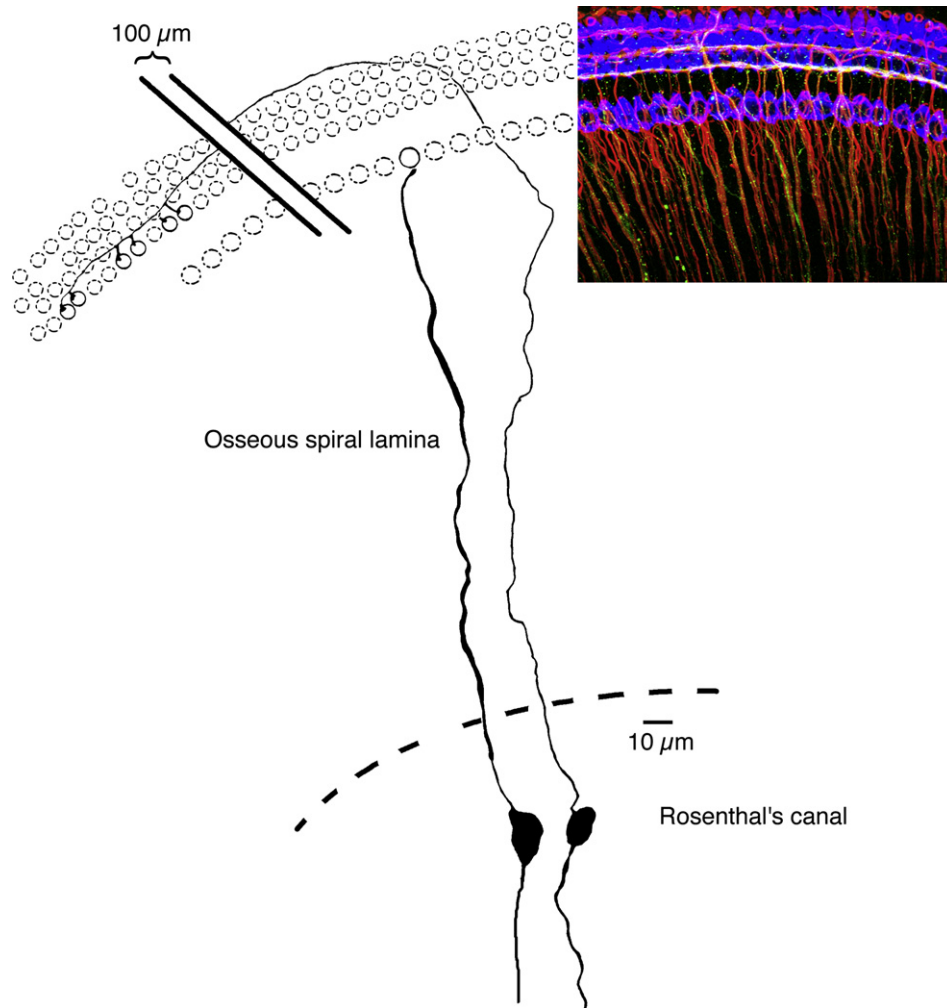


Fig. 6. Composite drawing tube reconstructions of HRP-labeled type I and type II neurons. The morphology of these neurons is representative of middle and basal turns of the mouse cochlea. Individual cell reconstructions are difficult but important because they enable the tracing of processes to their peripheral and central targets. (Modified from Berglund and Ryugo, 1987).

and gerbil, extracellular recordings and dye injections were conducted in the cochlear nucleus (Müller, 1990, 1991; Müller et al., 2005; Rivas et al., 2005). In these cases, labeled fibers were followed from the injection site back to their termination under IHCs in the cochlea and the longitudinal distance from the base determined. Normalized location was expressed as a percentage of the total length of the organ of Corti, and a simple logarithmic place-frequency map was established. The innervation patterns suggest that these animals are auditory generalists because they do not exhibit specialized innervation densities as has been reported for the horseshoe bat (Bruns and Schmieszek, 1980).

The size and location of the peripheral processes and terminal swellings are reflected by the spontaneous discharge rate (SR) and threshold of the fiber (Liberman, 1982; Liberman and Oliver, 1984). Processes from low and medium SR fibers (≤ 18 spikes/sec with higher thresholds) have thinner fiber diameters, low mitochondria content, terminate on the modiolar or ganglion side of the IHC, give rise to smaller terminal swellings, and have large synaptic ribbons. In contrast, fibers with high SR (> 18 spikes/sec with lower thresholds) have thicker fiber diameters, high mitochondria content, terminate on the pillar or tunnel side of the IHC, give rise to larger terminal swellings, and have small synaptic ribbons (Liberman, 1980, 1982; Liberman and Oliver, 1984; Merchan-Perez and Liberman, 1996). The question remains whether these

structural differences in the type I terminal that correlate with SR and threshold are causal or simply passive reflections of different overall activity levels. Regardless, these data reveal the three fundamental properties of auditory nerve fibers for type I spiral ganglion cells: SR, CF, and threshold. Given the segregation of these features to form distinct groupings, it may be inferred that the different classes of type I auditory nerve neurons serve different functions for hearing.

3.2. Type II neurons

The peripheral processes of type II ganglion cells are unmyelinated (Spoendlin, 1971, 1973; Romand and Romand, 1984, 1987). They course radially with the myelinated fibers before entering the cochlear duct through the habenula perforata (Fig. 6). The type I fibers turn upwards toward the IHCs, but the type II fibers cross the tunnel of Corti along its floor in a radial trajectory and then turn toward the basal end of the cochlea. These fibers in the organ of Corti were called outer spiral fibers (OSFs) before it was known that they were connected to type II neurons (Spoendlin, 1973, 1981; Smith, 1975). OSFs are relatively thin (< 1.0 μm), can spiral for hundreds of micrometers, and give rise to many large terminals under OHCs (Smith, 1973; Liberman and Simmons, 1985; Brown, 1987). Moreover, individual OSFs vary with respect to spiral

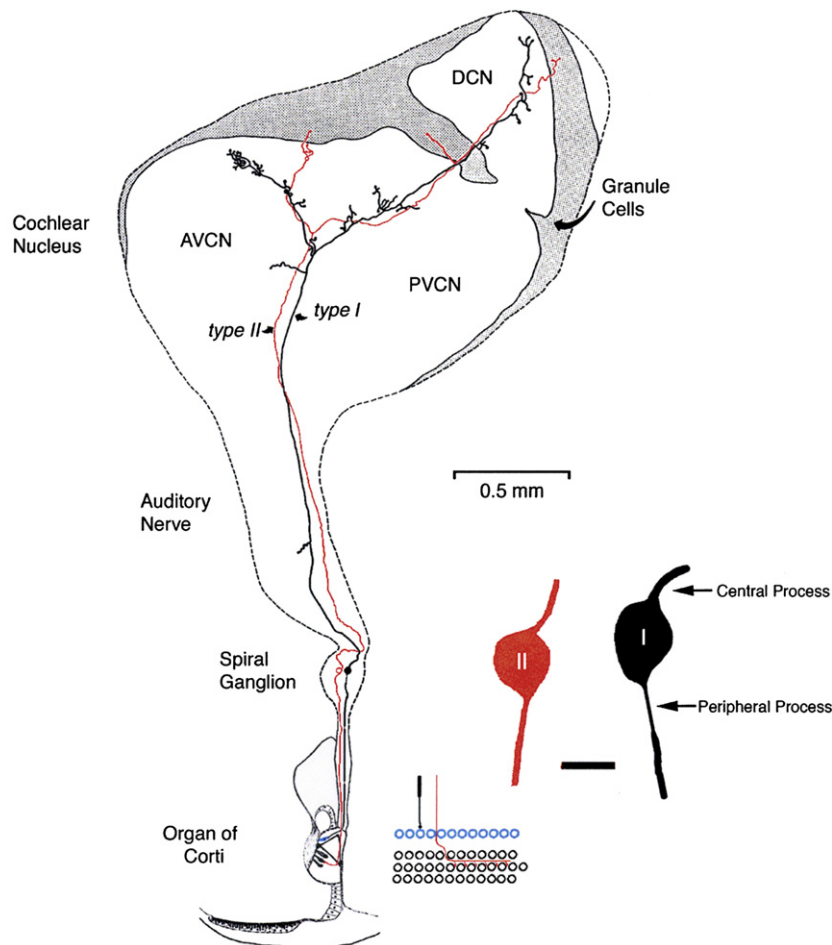


Fig. 7. Schematic drawing of spiral ganglion neurons and their central and peripheral terminations. The type I neuron (black) innervates a single inner hair cell and projects in a topographic fashion into the cochlear nucleus. Fibers that innervate the basal hair cells project to dorsal regions of the cochlear nucleus, whereas fibers that innervate more apical hair cells project to progressively more ventral regions. This type I neuron is representative of its group. Note that a representative type II neuron (red) has a similar central projection pattern but with additional terminations in the granule cell domain. (Adapted from Brown et al., 1988).

length, number of OHCs innervated, and row(s) of OHCs innervated. This basal trajectory is evident in the middle and basal ends of the cochlea but not necessarily for the apical region. Basal fibers tend to innervate OHCs within a single row.

In studies of the apex of the cochlea, OSFs exhibit a variety of different patterns: some were short and gave rise to terminals without spiraling, some spiraled in an apical direction, and some had complex branching patterns and contacted OHCs in all three rows (Perkins and Morest, 1975; Smith, 1975; Fechner et al., 2001). Apical type II fibers also can make more contacts with OHCs than basal ones, and these would include *en passant* contacts. One striking difference between apical and basal OSFs is that the apical fibers give rise to branches to supporting cells (Burgess et al., 1997; Fechner et al., 1998, 2001). OSFs that were traced back to type II cell bodies clearly branched to Hensen's and Deiters' cells of the organ of Corti (Fechner et al., 2001). These branches are more common in guinea pigs than in cats, and electron microscopic analysis of the branches suggest synaptic specializations that include prominent presynaptic membrane thickening, slight post-synaptic membrane thickening, and presynaptic accumulation of synaptic vesicles on the OSF side (Burgess et al., 1997). The implication is that OSFs maintain local circuit interactions between OHCs and supporting cells. It remains to be determined if there is mechanically evoked electrical activity in these supporting cells and if so, what the function might be.

The ultrastructural study of afferent synapses between type II terminals and OHCs revealed the presence of reciprocal synapses in

human, chimpanzee, cat, guinea pig, and mouse (Nadol, 1981; Francis and Nadol, 1993; Sobkowicz et al., 1993; Thiers et al., 2002, 2008). These synapses are found all along the length of the organ of Corti and in all three rows of OHCs. The afferent synapse was defined by pre- and post-synaptic membrane thickenings in association with a punctate narrowing of the intermembrane space (synaptic cleft) and accumulation of small vesicles (35–100 nm in diameter). Type II efferent synapses were characterized by a small cluster of vesicles within the OSF terminal and a prominent facing subsurface cistern within the OHC (Thiers et al., 2008). Exactly what these synapses are doing in terms of function is a complete mystery. Recordings at the type II afferent synapse reveal that release of synaptic vesicles by OHCs produces a relatively small depolarization (Weisz et al., 2009). Given the unmyelinated axon and the distance to the cochlear nucleus, it seems unlikely that this input by itself will directly signal auditory information. It seems more plausible that these relationships indicate a local circuit that enables communication among interconnected OHCs that modulates the output of the organ of Corti.

The two types of ganglion cells differ markedly in their cytologic and process features. Moreover, they differentially innervate the two types of hair cell receptors (Fig. 4), and they appear to have overlapping as well as different targets in the cochlear nucleus (Fig. 5). These data are highly suggestive that the separate populations of ganglion cells have different functions in acoustic processing.

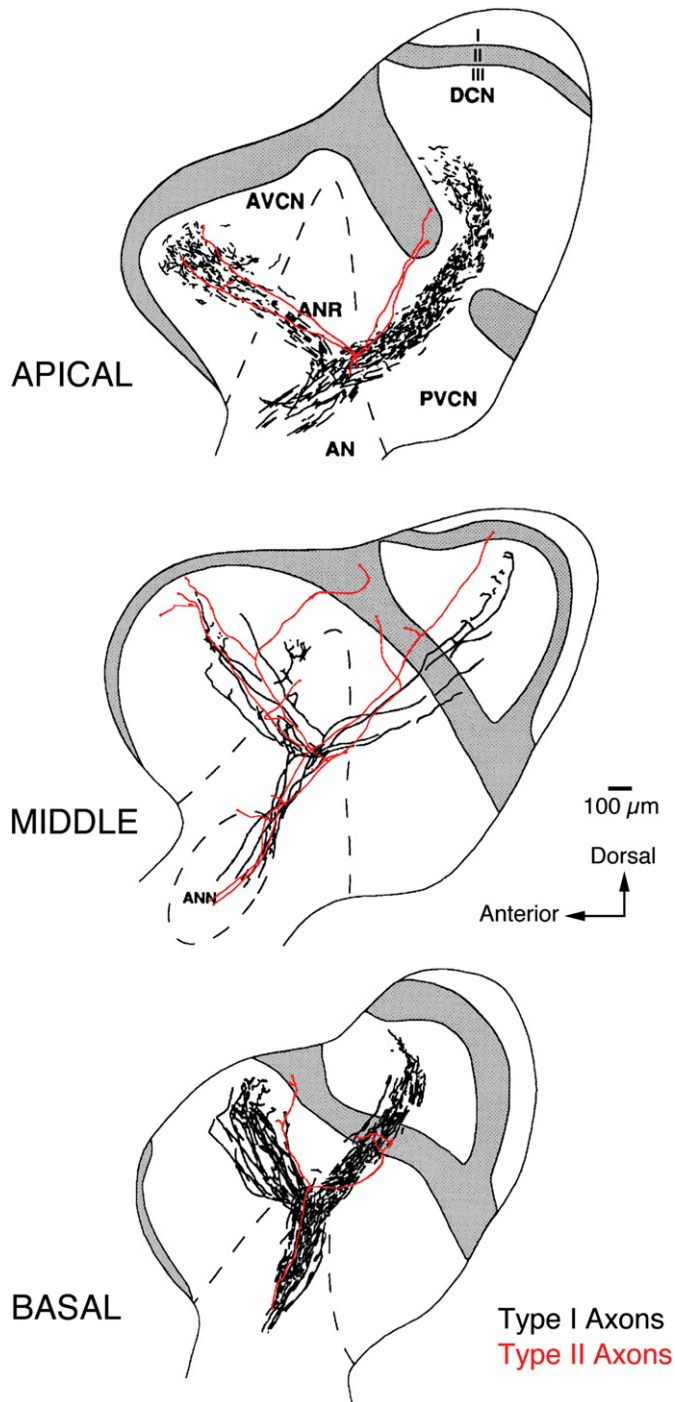


Fig. 8. Reconstructions of the cochlear nuclei from three separate mice, as viewed in sections collected parallel to the lateral surface of the nucleus and collapsed into a single plane. The labeled type I (black) and type II (red) fibers resulted from HRP injections into the different regions of the cochlea. Note that the fibers maintain their respective topography in their central projections with type II fibers having a preference for the granule cell domain. Abbreviations: AN, auditory nerve; ANN, auditory nerve nucleus; ANR, auditory nerve root; AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus. (Adapted from Berglund and Brown, 1994).

4. Central projections of the spiral ganglion to the cochlear nucleus

The central axons of spiral ganglion cells bundle together to form the modiolar segment of the auditory nerve. The nerve fibers run through the center of the cochlea, pass through the internal

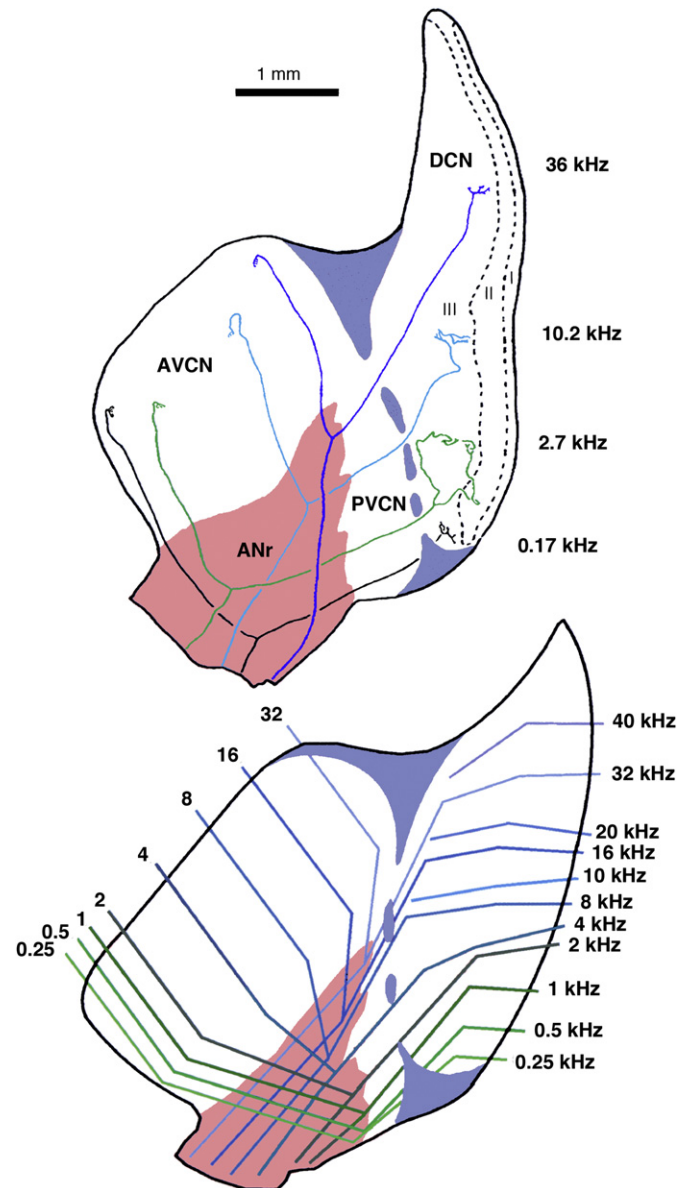


Fig. 9. (Top) Cochleotopic projections of type I auditory nerve fibers in cat as shown for the left cochlear nucleus in a side view. These fibers were stained using intracellular recording and dye injections, and reconstructed through serial sections. (Bottom) The projection reflects the tonotopic organization of neurons of the cochlear nucleus (Bourk et al., 1981). The correspondence of AN projections to the tonotopic organization of the AVCN implies that AN fibers and their terminations establish tonotopy in the nucleus. Abbreviations: ANR, auditory nerve root; AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus. (From Ryugo and Parks, 2003).

auditory meatus, and approach the cochlear nucleus from a ventral aspect (Figs. 7 and 8). Individual fibers enter the cochlear nucleus at the Schwann-glia border. The axons of both type I and type II spiral ganglion neurons ascend into the cochlear nucleus and bifurcate (Figs. 7 and 8). The position of the bifurcation is determined by the position of the termination in the organ of Corti that, in turn, determines the characteristic frequency (CF) of the fiber (Liberman, 1982; Fekete et al., 1984). The bifurcation creates an ascending branch that projects through the anteroventral cochlear nucleus (AVCN), and a descending branch that passes through the posteroventral cochlear nucleus (PVCN) to terminate in the dorsal cochlear nucleus (DCN). Short collateral branches are given off along the trajectory of the ascending and descending branches.

The distribution of the projecting fibers and collaterals exhibit features that are correlated to their physiological properties. First, there is a distinct frequency organization to the projection (Fig. 9). Extending away from the frequency-dependent bifurcation, fibers innervate the cochlear nucleus such that apical, low frequency fibers are distributed ventrally and progressively, higher frequency fibers are distributed progressively more dorsally (Figs. 8 and 9). The collateral branches, with a few exceptions that will be discussed later, do not extend far from the parent branch. These branches divide several times and exhibit *en passant* and terminal swellings. The *en passant* swellings may or may not exhibit membrane specializations typical of synapses, but the terminal swellings are sites of synapses (Fekete et al., 1984; Ryugo et al., 1991). The spatial distribution of terminals from individual fibers exhibit what may be described as a patchy, incomplete ribbon. It is inferred that collections of fibers having similar frequency responses distribute terminals that fill in the patches to form a complete ribbon, and that collections of ribbons form iso-frequency laminae (Fig. 10).

The portion of the auditory nerve fiber prior to the bifurcation is called the root branch. Collaterals can arise from the root branch although they are present on fewer than half the fibers. These collaterals are obviously thinner than the parent branches (e.g., ascending, descending, and root branches) so they are readily distinguishable and are not confused with the bifurcation. Their numbers, lengths, and additional branching features vary from fiber to fiber, and are more common among fibers with higher CFs. The

intermingling of root, ascending, and descending branches and their collaterals (Fig. 10) complicates the frequency organization in the auditory nerve root region (Bourk et al., 1981; Fekete et al., 1984).

Morphological specializations of the central projections have also been shown to be related to fiber SR. There is spatial segregation of spiral ganglion neurons within the ganglion with respect to SR: low SR neurons tend to be located on the scala vestibuli side of Rosenthal's canal, whereas high SR neurons are found throughout the canal (Kawase and Liberman, 1992; Leake et al., 1993). This trend is continued as the specializations become superimposed upon the tonotopic organization of the projection. While the main ascending and descending branches of auditory nerve fibers are remarkably similar in distribution (Fig. 11), the low SR fibers give rise to substantially larger axonal arborizations within the AVCN compared to that of high SR fibers (Fekete et al., 1984). Note especially the relatively long, elaborate branches that are emitted by low SR fibers into the peripheral borders of the cochlear nucleus, specifically the small cell cap (Liberman, 1991; Ryugo, 2008). This cap of small cells resides subjacent to the granule cell domain, and is most evident along the dorsal, lateral, and dorsomedial aspect of the AVCN (Osen, 1969). The collaterals of low SR fibers are distributed primarily within the dorsal and lateral regions of the cap, but can occasionally be found in the medial zone (Fig. 12). These numerous small terminals selectively target the somata and dendrites of small cells of the cap (Ryugo, 2008).

The relationship of high threshold, low SR auditory nerve projections into the small cell cap has functional relevance.

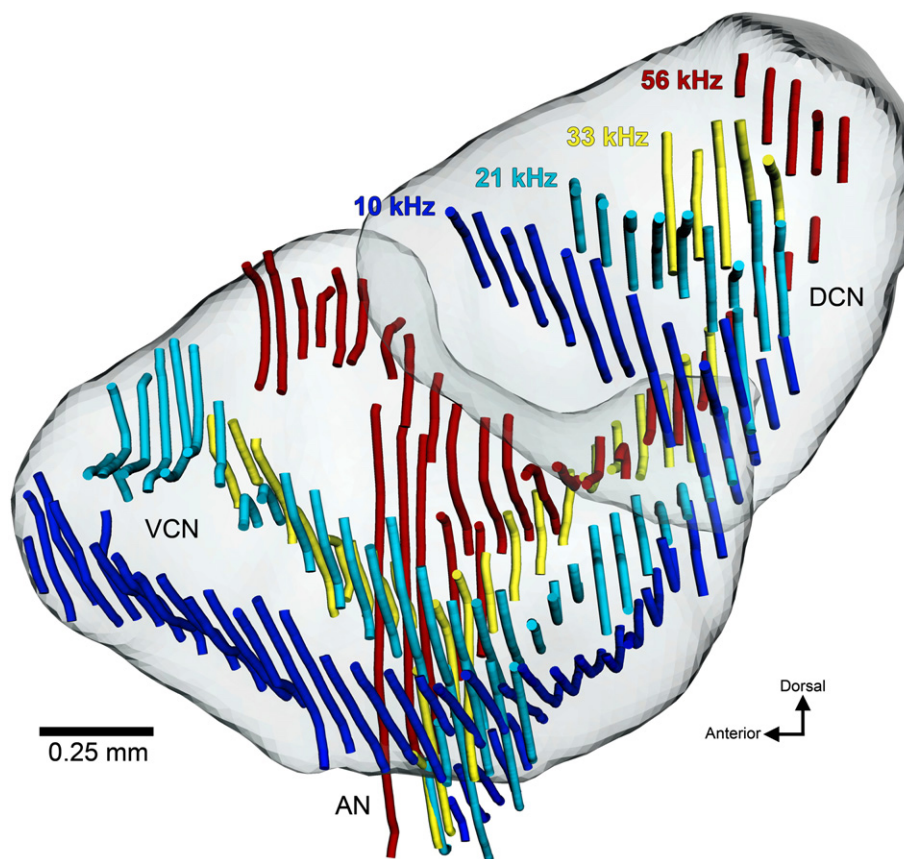


Fig. 10. Cochleotopic projection of auditory nerve fibers in mouse as shown in a lateral view for the left cochlear nucleus reconstructed from serial sections. Auditory nerve fibers were labeled with fluorescent dextran amine after extracellular multiunit characterization of best frequency. Each stripe (or noodle) represents the extent of labeling within a single section. A three-dimensional atlas of the nucleus was made and projections from four different mice were inserted (Muniak et al., 2011). Note the creation of spatially separate “isofrequency sheets” by the linking of individual stripes. This organization holds true for much of the nucleus but note how the AN root region is complicated by all the intermingling of fibers of different frequencies.

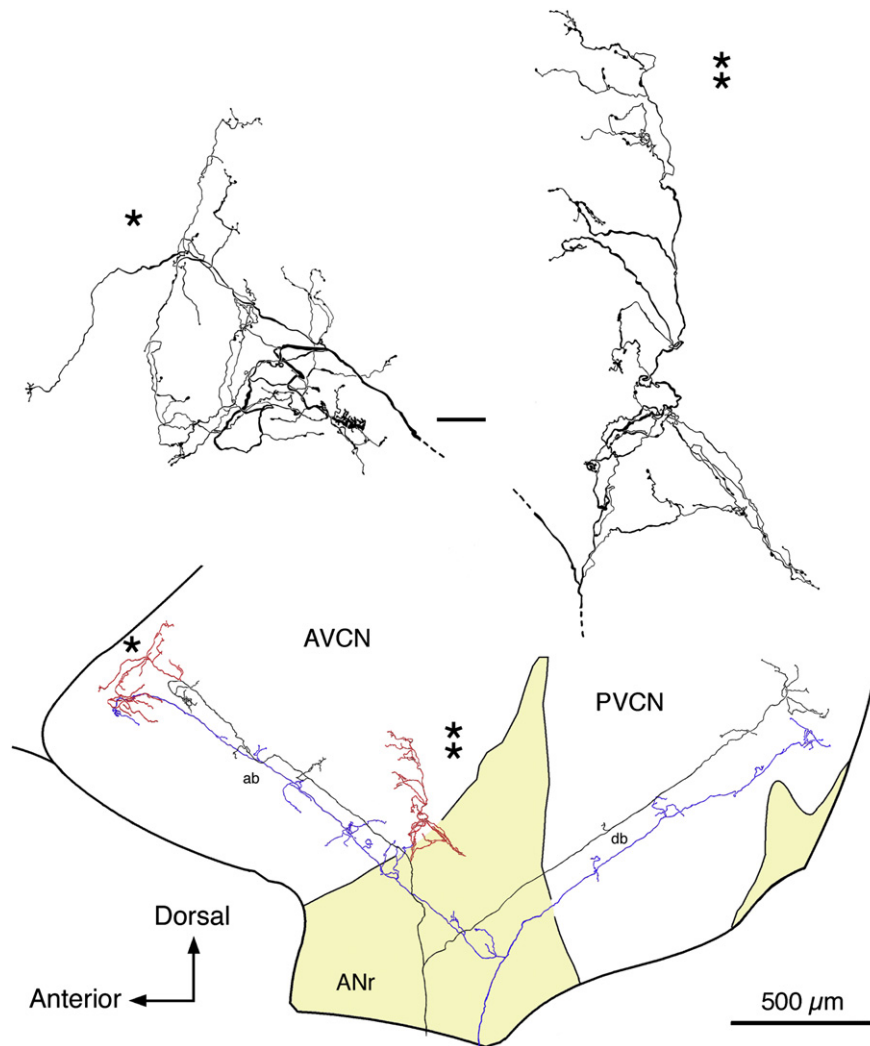


Fig. 11. Drawing tube reconstructions of a low SR auditory nerve fiber (black and red, CF = 3.1 kHz; SR = 0.2 s/s; Th = 26 dB SPL) and a high SR auditory nerve fiber (blue, CF = 1.2 kHz, SR = 86 s/s; Th = −3 dB) as viewed laterally. The ascending branches take a relatively straight trajectory through the AVCN. Low SR fibers are distinctive by the collaterals that arborize within the small cell cap (red). Otherwise, the main parts of the ascending and descending branches are similar for the different SR fiber types (black, low SR; blue, high SR). Higher magnification drawings are shown for each collateral. One collateral ramifies anterior to the endbulb (*), whereas the other ramifies laterally (**). The collaterals of high threshold, low SR fibers ramify extensively within the small cell cap and are good candidates for serving as the afferent limb of the high threshold circuit that feeds back to the organ of Corti by way of the olivocochlear system (Ye et al., 2000). Abbreviations: ab, ascending branch; ANr, auditory nerve root; AVCN, anteroventral cochlear nucleus; db, descending branch; PVCN, posteroventral cochlear nucleus. The scale bar equals 25 μ m for the high magnification collateral drawings (top) and 500 μ m for the orientation drawing (bottom). (Adapted from Fekete et al., 1984 and Ryugo, 2008).

Evoked activity in these fibers by loud sounds would not only suggest that a large pool of auditory nerve fibers has been activated, but also that this activity would be distributed across numerous small cells in the cap. Cells of the cap exhibit high thresholds (Ghoshal and Kim, 1996, 1997) so their recruitment would be consistent with the notion that they encode aspects about the intensity of the acoustic stimulus. Moreover, the small cell cap has been shown to send axons to the dendrites and somata of neurons belonging to the medial olivocochlear efferent system (Ye et al., 2000). In this context, the low SR auditory nerve fibers, cells of the small cell cap, and medial efferent olivocochlear neurons represent separate links of a high threshold feedback circuit to the inner ear.

A large, axosomatic terminal, called the endbulb of Held, marks the end of the ascending branch in the AVCN (Held, 1893; Brawer and Morest, 1975; Lorente de N , 1981; Ryugo and Fekete, 1982), and is noteworthy for several reasons. This ending is remarkable for its size and its evolutionary conservation: they have been observed in every vertebrate examined to date (Fig. 13), suggesting functional

significance and survival advantages (Ryugo and Parks, 2003). In cats, it has been estimated that a single endbulb can contain up to 2000 synaptic release sites (Ryugo et al., 1996), implying that transmission from endbulb to post-synaptic neuron would occur with high fidelity. The secure transmission would ensure that neural activity is tightly coupled to acoustic events, permitting the system to utilize interaural timing cues to localize sound in space (Pfeiffer, 1966; Smith et al., 1993). Moreover, this ending shows an impressive degree of plasticity in terms of its shape and the structure of its synapses.

The endbulbs of normal hearing animals have highly elaborate arborizations. Postnatally, however, they appear as club-shaped structures that pass through a reticulated stage and finally transform themselves into the mature form [Fig. 14, (Ryugo and Fekete, 1982; Limb and Ryugo, 2000)]. If, however, the animal is congenitally deaf, the endbulbs develop into a less branched, more truncated form of the ending (Fig. 15); they are still recognizable as endbulbs, but their structural complexity is greatly reduced (Ryugo et al., 1997, 1998). Normally, endbulb synapses exhibit discrete, dome-shaped

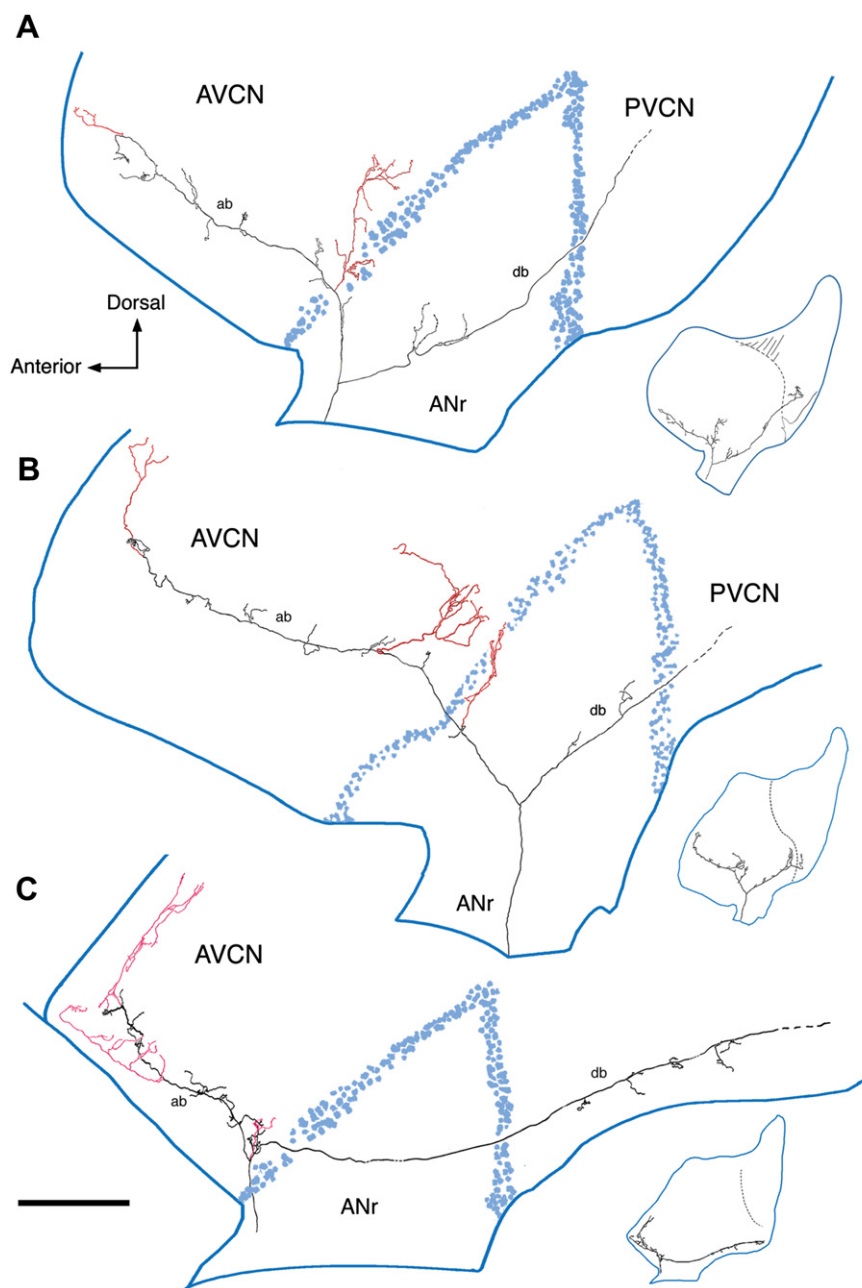


Fig. 12. This figure presents three reconstructed low SR fibers in the cochlear nucleus in side view. The collaterals that innervate the small cell cap rostrally and laterally are shown in red. (A) CF = 1.2 kHz, SR = 1.0 s/s, Th = 4 dB SPL; (B) CF = 1.85 kHz, SR = 0 s/s, Th = 50 dB SPL; (C) CF = 0.3 kHz, SR = 0.1 s/s, Th = 39 dB SPL. The distributed terminals from these collaterals have areal spread over the nucleus yet are also confined to a thin zone squeezed between the outermost GCD and the underlying magnocellular core. Since low SR fibers have high thresholds, their activation by intense sounds would tend to produce a divergent spread of activity to neurons of the small cell cap. This divergence would recruit additional neurons, which could be important because for many AN fibers, increased sound level does not result in increased spike rates (Kiang et al., 1965). Abbreviations: ab, ascending branch; ANr, auditory nerve root; AVCN, anteroventral cochlear nucleus; db, descending branch; PVCN, posteroventral cochlear nucleus. Scale bar equals 0.5 mm (From Ryugo, 2008).

post-synaptic densities (PSDs; Fig. 16). With deafness, however, the synapses are abnormal exhibiting flat and hypertrophied PSDs with an increased density of synaptic vesicles [Fig. 17, (Ryugo et al., 1997; Lee et al., 2003; O'Neil et al., 2010)]. These changes may be related to physiological reports of increased synaptic strength in the AVCN of congenitally deaf mice when compared to hearing mice (Oleskevich and Walmsley, 2002; Oleskevich et al., 2004). The increase in synaptic currents could be a result of the increased number of synaptic vesicles and an increase in transmitter receptors that accompany the PSD hypertrophy.

Remarkably, the abnormal morphology of endbulbs and their synapses in congenitally deaf cats can be restored to near-normal

by introducing auditory nerve activity by way of cochlear implants to three-month old kittens [Fig. 18, (Ryugo et al., 2005; O'Neil et al., 2010)]. If activity was introduced to six-month old kittens, however, there was no effect on endbulb structure. These data support the idea that there is a critical period for rehabilitation, and that the developmental period preceding puberty is optimal for activity-related synaptic plasticity. We conclude that auditory nerve synapses are structurally plastic with respect to activity, where size and shape appear related to activity levels (Rees et al., 1985; Ryugo et al., 1998; O'Neil et al., 2010). The extent to which structural plasticity underlies functional plasticity remains to be determined.

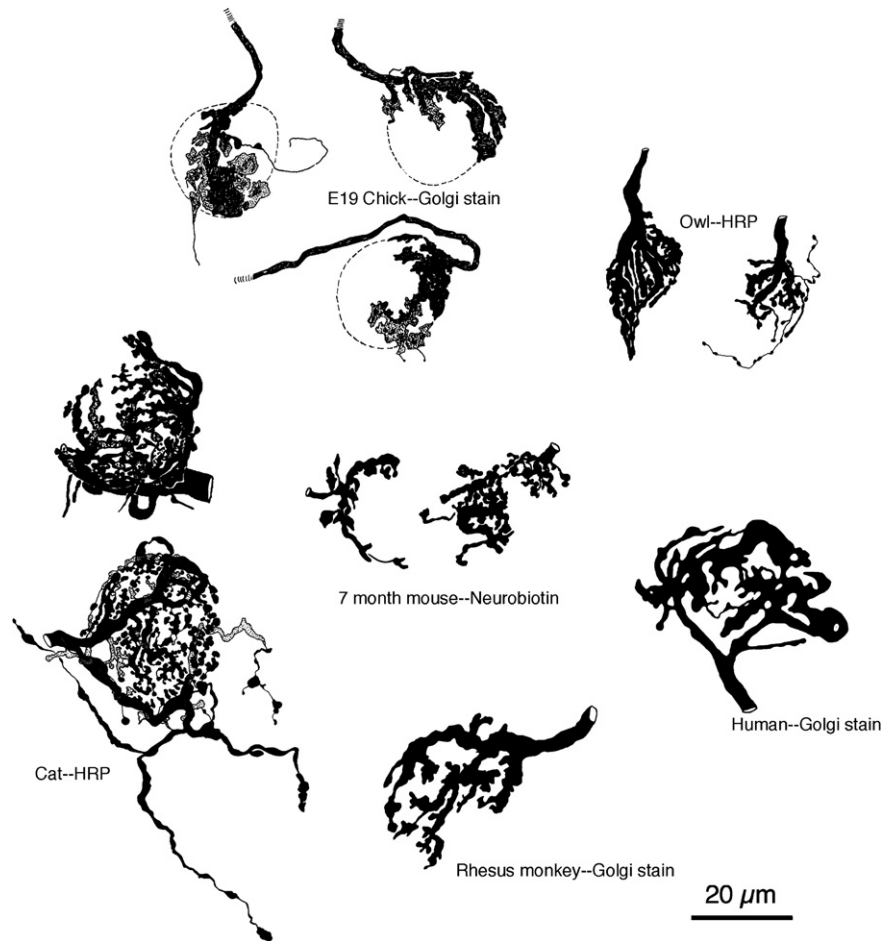


Fig. 13. Large axosomatic endings are formed by auditory nerve fibers across a wide variety of animals. Their size implies a powerful influence upon the post-synaptic neuron, and their function has been inferred to mediate precise temporal processing. The evolutionary conservation further argues for survival advantages conferred by accurate timing information for sound localization acuity and auditory discrimination skills. Chick endbulbs are from [Jhaveri and Morest \(1982\)](#), Plenum Press; owl endbulbs are from [Carr and Boudreau \(1996\)](#), Wiley-Liss Publishers; mouse endbulbs are from [Limb and Ryugo \(2000\)](#), Springer-Verlag Publishers; cat endbulbs are from [Ryugo et al. \(1998\)](#), Wiley-Liss Publishers, and [Sento and Ryugo, 1989](#), Alan R. Liss Publishers; monkey endbulb is from Ryugo, unpublished data; human endbulb is from [Adams \(1986\)](#), American Medical Association. (From [Ryugo and Parks, 2003](#)).

5. Differences in synaptic input to the somata of type I and II neurons

Type II ganglion cells in the human were shown to receive axosomatic synapses ([Kimura et al., 1979](#); [Thiers et al., 2000](#)). Similar

kinds of synapses with asymmetric membrane thickenings and round synaptic vesicles were also observed in macaque monkeys ([Kimura et al., 1987](#)). The presence of these synapses raises the possibility of efferent modulation of type II activity from neurons whose cell bodies reside in the superior olivary complex. There have

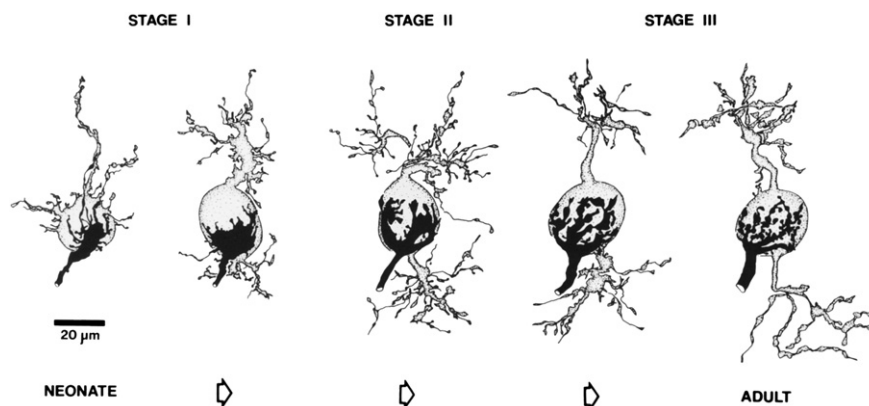


Fig. 14. Drawings that illustrate the inferred sequence of development for a single endbulb of Held and its post-synaptic spherical bushy cell. Stage I and II endbulbs are found during the first three post-natal weeks; the endbulb situated between stage II and III is 45 days of age; Stage III endbulbs are representative of 6 month old cats. The dramatic structural changes that occur with maturation emphasize the need to recognize age as an important variable when conducting experiments and interpreting data. (From [Ryugo and Fekete, 1982](#)).

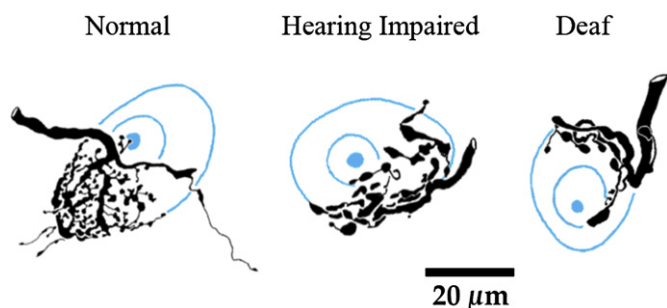


Fig. 15. Drawing tube reconstructions of endbulbs of Held from adult cats with normal hearing, early onset hearing loss, and congenital deafness. The loss of structural complexity as indicated by fractal value is graded and correlated to the degree of hearing loss (Ryugo et al., 1998).

also been reports of dendrodendritic synapses between type II fibers that could affect the nature of signaling between OHCs (Nadol, 1988; Spoendlin and Schrott, 1988; Nadol et al., 1990). Of particular interest is the demonstration of “mitochondria-associated adherence complexes” that are part of the synapses. This type of structure has been previously described (Gray, 1963; Cant and Morest, 1979; Spirou et al., 1998) and it has been associated with neurons that exhibit high rates of spike activity and synaptic vesicle turnover (Spirou et al., 1998; Rowland et al., 2000). The conflict is that type II neurons have very high thresholds to activation (Weisz et al., 2009) with no spontaneous or evoked activity to sound (Robertson, 1984).

The observations of synapses on cell bodies of spiral ganglion cells lies in sharp contrast to a failure to find such contacts in other mammals, including mouse (Romand and Romand, 1987), chinchilla (Ruggero et al., 1982), cat (Adamo and Daigneault, 1973), and guinea pig (Thomsen, 1966). Those studies that found somatic synapses used serial section analysis, whereas those that did not, with the exception of the cat, used random sampling techniques. Due to the small size of these synapses, the failure to find them could simply reflect a sampling limitation.

It may be safely concluded that in the mammalian spiral ganglion, there are two types of neurons: a class of large cells that innervate the IHCs and a class of small cells that innervate OHCs. The numbers of hair cells and ganglion neurons and the innervation density across a number of mammals are summarized in Tables 1 and 2. There are, however, variations within this theme when considering primates, particularly humans. In these species, the somata of spiral ganglion neurons tend not to be myelinated, and this feature allows access for synaptic terminals. If the spiral ganglion cell body in primates acts like a node of Ranvier, however, perhaps the human spiral ganglion is functionally not all that different from that of other mammals.

6. Potential for plasticity and repair

6.1. Neurotrophins, development, and survival of spiral ganglion neurons

The neurotrophins form a large family of proteins that act via tyrosine receptor kinase (Trk) signaling to promote the survival of neurons. *In situ* hybridization studies have implicated two neurotrophins in normal ear development and function: brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) (Pirvola et al., 1992). Specifically, these authors illustrated that the mRNAs for both BDNF and NT3 were expressed in overlapping and distinct regions in the developing otic vesicle and were localized to the sensory epithelium later in development. This result was further substantiated by the localization of both BDNF and NT3 mRNA in the developing and early post-natal organ of Corti concomitant with the expression of mRNA for their respective tyrosine receptor kinases, TrkB and TrkC, in the spiral ganglion (Ernfors et al., 1992; Ylikoski et al., 1993). The lack of expression of either nerve growth factor (NGF) or neurotrophin-4/5 (NT4/5) mRNAs in these studies suggested a specific role for BDNF and NT3 in inner ear development (Ernfors et al., 1992; Pirvola et al., 1992; Ylikoski et al., 1993).

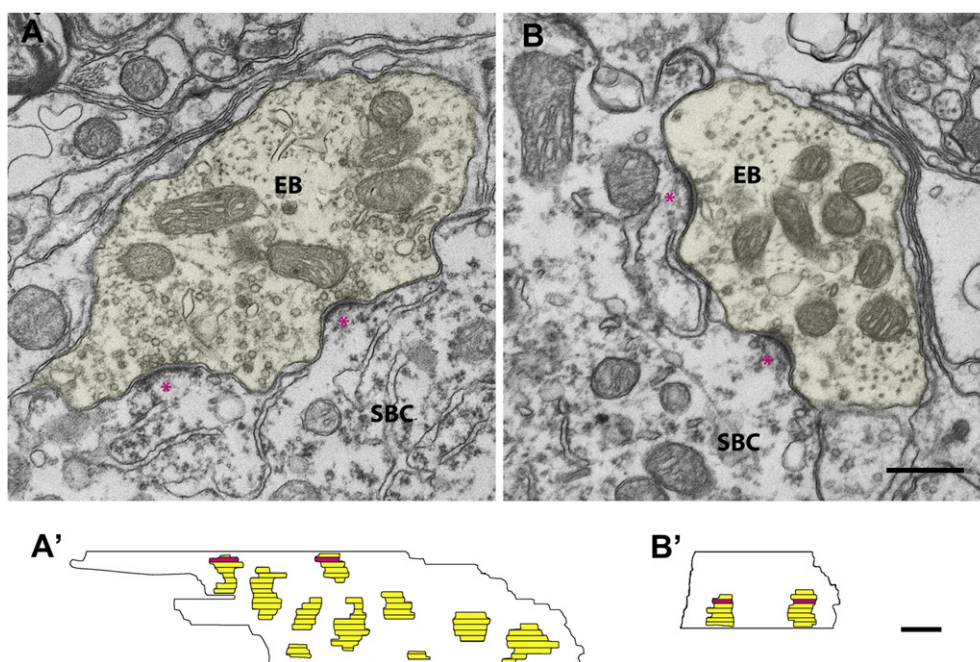


Fig. 16. Electron micrographs of endbulbs of Held (EB, highlighted in yellow) from normal hearing cats (A, B). The micrographs show the typical dome-shaped appearance of PSDs (*) and associated synaptic vesicles. The drawings (A'–B') display corresponding *en face* views of the three-dimensional reconstructions, illustrating the surface of the SBC that lies beneath the endbulb. Each PSD from serial sections (A', B') is shown (gray) and horizontal lines indicate section edges. The darker lines highlight the section of the EB series shown in the electron micrographs. Scale bars equal 0.5 μ m (From O'Neil et al., 2010).

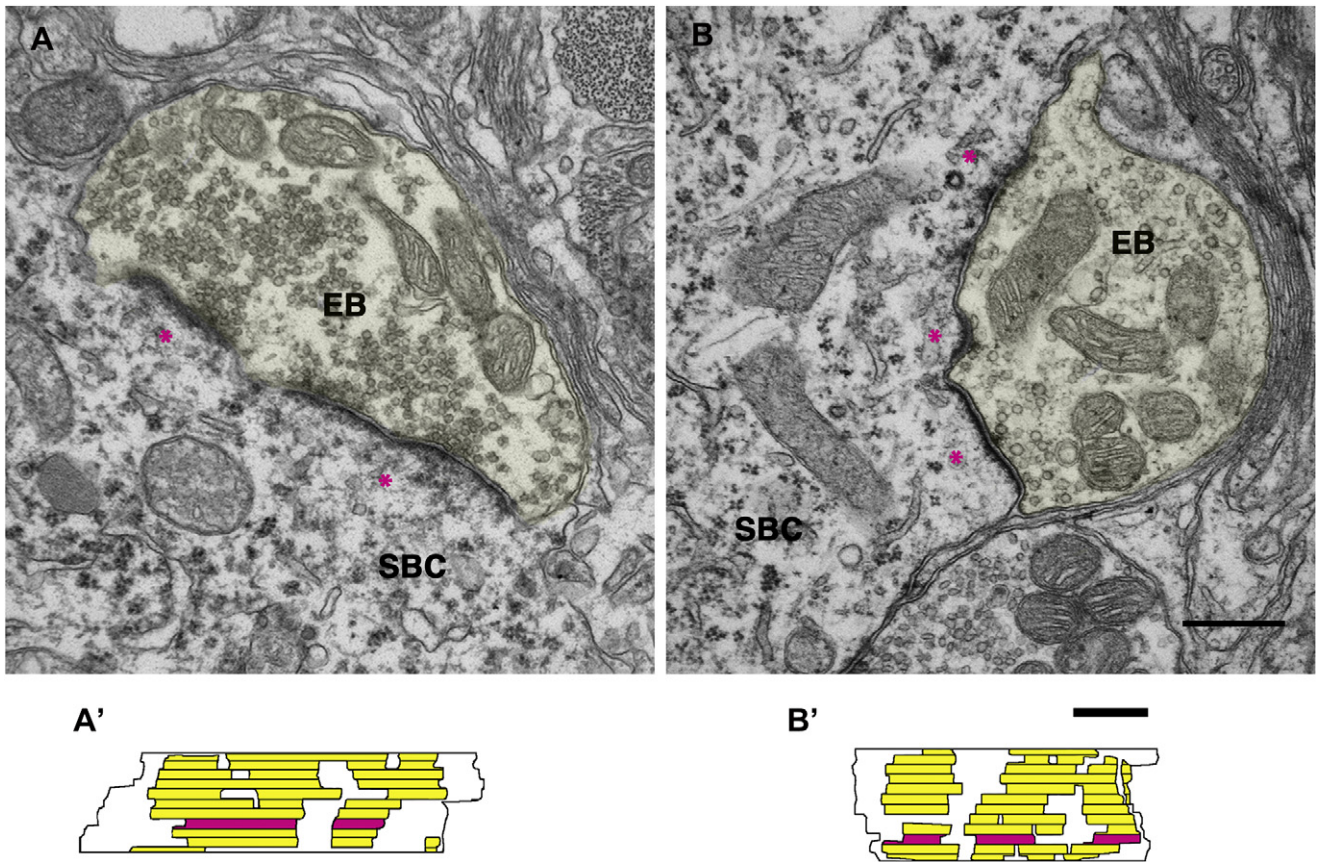


Fig. 17. Electron micrographs of EB profiles (yellow) from congenitally deaf cats (A, B). These endbulbs display the abnormally long and flattened PSDs (*) and heightened clustering of associated synaptic vesicles. The three-dimensional reconstructions (A', B') illustrate the hypertrophy of the PSDs (gray). The horizontal lines mark the section edges and the dark strip highlights the section of the EB series shown in the above micrographs. Scale bars equal 0.5 μm (From O'Neil et al., 2010).

The complementary roles of BDNF and NT3 and their high affinity Trk receptors in auditory development have now been thoroughly investigated in animal knock-out studies where genes encoding either the neurotrophins or their receptors were deleted (Table 3). For example, mice devoid of NT3 show a significant reduction (85%) in the number of spiral ganglion neurons formed, in comparison to wild-type controls (Farinas et al., 1994). Conversely, BDNF mutants show a loss of more than 80% of vestibular ganglion neurons, which is accompanied by deficiencies in balance and co-ordination (Ernfors et al., 1994). Using BDNF, NT3 and combined BDNF/NT3 deletions in the developing inner ear it was shown that BDNF mutants lost 79% of vestibular neurons and 7% of spiral ganglion neurons, whereas NT3 mutants lost 87% of spiral ganglion neurons and 34% vestibular neurons. Double mutants, with both BDNF and NT3 genes deleted, were the most severely compromised, lacking almost all vestibular and spiral ganglion neurons at birth (Ernfors et al., 1995).

Animals lacking the Trk receptors for these neurotrophins displayed a similar trend in spiral ganglion neuron loss. For example, there was a 15% reduction in the number of spiral ganglion neurons present at birth following TrkB receptor deletion, but a 56% reduction in vestibular ganglion neurons. In comparison, TrkC receptor deletion resulted in a 51% reduction in spiral ganglion neurons and a 16% loss of vestibular neurons at birth (Schimmang et al., 1995). Deletion of both TrkB and TrkC receptors resulted in a loss of 58% and 61% of vestibular and cochlear ganglion neurons, respectively (Minichiello et al., 1995). The differences in percentage loss between TrkC mutants and NT3 mutants has been attributed to the limited ability of NT3 to signal through the TrkB receptor (Fritzsche et al., 1997b). In later experiments

using a full length null mutation to TrkC, the loss of basal turn afferent innervation was comparable to that observed in NT3 mutants (Tessarollo et al., 1997). Collectively, these studies illustrated the essential and defined roles of BDNF and NT3 in auditory development and the reliance of spiral ganglion neurons upon neurotrophic support from the developing organ of Corti.

These studies also suggested defined roles for each neurotrophin in the afferent innervation of the cochlea (Ernfors et al., 1995; Minichiello et al., 1995; Schimmang et al., 1995). The differential effects of BDNF and NT3 gene deletion on the survival of spiral ganglion neurons led to the conclusion that BDNF was the likely survival factor for type II spiral ganglion neurons (OHC innervation) and NT3 for type I spiral ganglion neurons (IHC innervation) (Ernfors et al., 1995). This idea was consistent with suggestions that OHC innervation was supported by BDNF/TrkB, whereas IHC innervation was dependent upon NT3/TrkC (Minichiello et al., 1995; Schimmang et al., 1995).

In a subsequent more detailed study using tracing analysis, it was revealed that there was a selective region-specific loss of spiral ganglion neurons in NT3 null mutant mice. In this study, all spiral ganglion neurons were lost in the basal turn of the cochlea, in comparison to the middle and apical turns where partial survival of both IHC and OHC innervation was observed (Fritzsche et al., 1997a). The observed gradient of neuronal dependency on NT3 was consistent with the basal-to-apical expression profile of this neurotrophin during normal development (Pirvola et al., 1992). Alternatively, while BDNF mutants showed a complete loss of OHC innervation in the apical turn of the cochlea (Ernfors et al., 1995), innervation of OHCs was observed in the basal turn of these

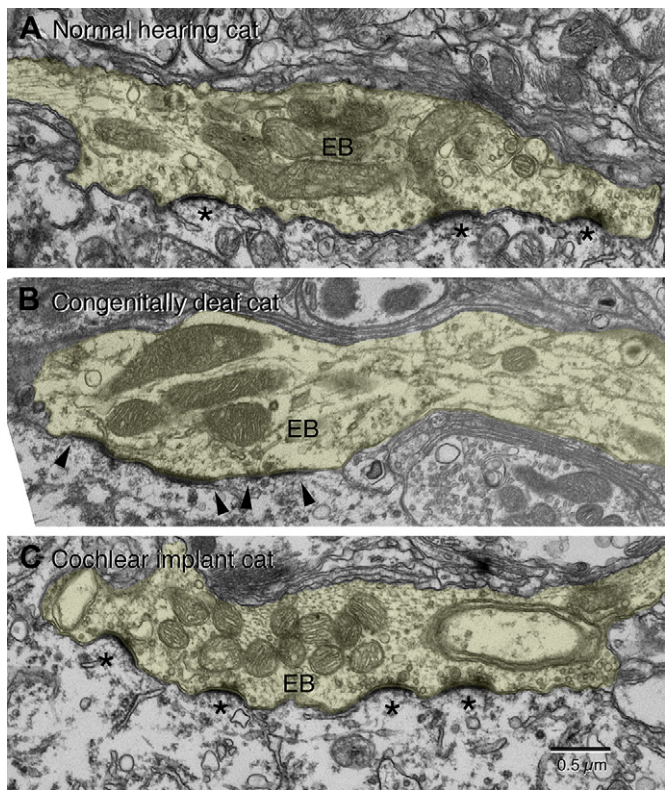


Fig. 18. Electron micrographs of endbulb (EB) synapses from (A) a normal hearing cat, (B) a congenitally deaf cat; and (C) a congenitally deaf cat that received approximately 3 months of electrical stimulation from a cochlear implant. All micrographs were collected from cats that were 6 months of age. Note that the synapses of the cochlear implant cat are restored to near normal as they are punctate, curved and accompanied by a normal complement of synaptic vesicles (asterisks). Scale bar equals 0.5 μ m (From Ryugo et al., 2005).

animals (Bianchi et al., 1996; Fritzsche et al., 1997a, 1997b). These data suggested that spiral ganglion neuron survival is dependent upon location in the cochlea, rather than spiral ganglion cell type. During auditory development, NT3 is observed to exert its greatest influence on basal spiral ganglion neurons, whereas BDNF acts preferentially on apically located spiral ganglion neurons (Pirvola et al., 1992; Farinas et al., 2001). This period is followed by high NT3 expression in the apex of the post-natal cochlea (Pirvola et al.,

Table 1
Counts of IHCs, OHCs, and SGNs.

Species	IHCs	OHCs	SGNs	Reference
Echidna	2700	5050		Ladhams and Pickles, 1996
Platypus	1600	3350		Ladhams and Pickles, 1996
House Mouse	765	2500	12,350	Ehret, 1983
Rat	960	3470	15,800	Keithley and Feldman, 1979;
<i>Sprague–Dawley</i>			14,305	Berglund and Ryugo, 1991;
			19,229 \pm 1049	Hall and Massengill, 1997
Guinea Pig		2400	24,011 \pm 982	Gacek and Rasmussen, 1961;
			19,323 \pm 1186	Firbas et al., 1970;
				Nadol, 1988
Cat	3000	9000	50,000	Retzius, 1884;
		9900	48,957 \pm 1274	Held, 1926;
			50,558 \pm 5285	Chen et al., 2010;
			51,574 \pm 2933	Howe, 1934;
				Gacek and Rasmussen, 1961;
Squirrel Monkey			30,745 \pm 2769	Alving and Cowan, 1971;
Rhesus Monkey			31,247 \pm 2114	Gacek and Rasmussen, 1961;
Human	3700	14,600	30,000	Retzius, 1884;
				Rasmussen, 1940;
				Bredberg, 1968

Table 2
IHC and OHC Innervation Density.

Species	IHCs Innervation Density	OHCs Innervation Density	Reference
Mouse	13		Ehret and Frankenreiter, 1977; Ehret, 1979;
Guinea Pig	10	5–15	Keithley and Feldman, 1979
Horseshoe Bat	8–24	3	Firbas, 1972;
Cat	10–26	4–20	Smith and Sjöstrand, 1961 Brunns and Schmieszek, 1980
Little Brown Bat	70		Gacek and Rasmussen, 1961; Spoendlin, 1969, 1973;
Human	8–11		Lieberman, 1980; Keithley and Schreiber, 1987; Simmons and Liberman, 1988; Lieberman et al., 1990 Ramprashad et al., 1978

1992; Ylikoski et al., 1993; Fritzsche et al., 1997a). At birth the expression of these neurotrophins is reported to be relatively even. The mirror image neurotrophin gradient model predicts that BDNF expression will be highest in the base of the adult cochlea (Davis, 2003), although this relationship remains to be elucidated.

6.2. Neurotrophins and spiral ganglion survival *in vitro* and *in vivo*

Numerous studies have reported the survival-promoting effect of exogenous neurotrophins on early post-natal rat spiral ganglion neurons *in vitro*. BDNF (Malgrange et al., 1996; Zheng and Gao, 1996; Marzella et al., 1999; Gillespie et al., 2001), NT3 (Marzella et al., 1997) and neurotrophin-4/5 (NT4/5) (Zheng and Gao, 1996) have all been shown to enhance spiral ganglion neuron survival in comparison to control treatments *in vitro*. In addition, transforming growth factor- β 3 and - β 5 were shown to promote spiral ganglion neuron survival *in vitro* in a concentration dependent manner (Marzella et al., 1999). Neurotrophins have been reported to act synergistically with one another (Marzella et al., 1999) and in combination with the neuronal cytokines leukemia inhibitory factor (LIF) or ciliary derived neurotrophic factor (CNTF) (Staecker et al., 1995; Hartnick et al., 1996; Marzella et al., 1997, 1999; Gillespie et al., 2001; Whitlon et al., 2006), to enhance the survival of post-natal spiral ganglion neurons *in vitro*. Recently, it was shown that the ratio of bipolar to monopolar spiral ganglion neurons could be controlled using defined culture conditions containing LIF and CNTF (Whitlon et al., 2007). Cultures of adult rat spiral ganglion neurons have also shown significantly enhanced survival following the application of exogenous BDNF and NT3 (Lefebvre et al., 1994). Together, these results reiterate the

Table 3
Summary of neurotrophin and neurotrophin receptor disruption in the mammalian cochlea: affect on spiral ganglion neuron survival.

Knock-out	Cochlear spiral ganglion neurons; percentage reduction after birth	Reference
BDNF	7%	Ernfors et al., 1995
NT3	85%	Farinas et al., 1994
	87%	Ernfors et al., 1995
BDNF/NT3	100%	Ernfors et al., 1995
TrkB	15%	Schimmang et al., 1995
TrkC	51%	Schimmang et al., 1995
	70%	Tessarollo et al., 1997
TrkB/TrkC	61%	Minichiello et al., 1995

requirement of sustained neurotrophic support for spiral ganglion neuron survival during development and throughout adulthood.

Nerve growth factor (NGF), BDNF, NT3, NT4/5, CNTF and glial cell derived neurotrophic factor (GDNF) have all been shown to prevent spiral ganglion neuron degeneration following sensorineural hearing loss *in vivo* (Shah et al., 1995; Ernfors et al., 1996; Staecker et al., 1996, 1998; Miller et al., 1997; Ylikoski et al., 1998; Kanzaki et al., 2002; Gillespie et al., 2003, 2004; McGuinness and Shepherd, 2005; Richardson et al., 2005; Shepherd et al., 2005; Wise et al., 2005). This protective effect was observed following treatment periods of two to eight weeks in mammals, with significantly greater numbers of spiral ganglion neurons reported in neurotrophin-treated cochleae (in comparison to untreated controls) even after eight weeks of treatment (Staecker et al., 1996). Furthermore, the protective effects of neurotrophins were observed after delayed infusion. For example, separate intracochlear delivery of BDNF, NT3, NT4/5 and NGF all prevented further degeneration of spiral ganglion neurons following a two-week period of deafness (Gillespie et al., 2004).

A similar protective effect was observed when a combination of BDNF and NT3 was infused following a four-week period of deafness (Richardson et al., 2005; Wise et al., 2005). Although neurotrophin infusion was capable of a protective effect six weeks post-deafening, the degree of spiral ganglion neuron survival was reduced in comparison to cochleae treated identically two weeks post-deafening (Yamagata et al., 2004). This observation suggests that neurotrophic rescue of spiral ganglion neurons may be of limited effectiveness after prolonged periods of deafness due to the ongoing degeneration of spiral ganglion neurons. However, the slower rate of spiral ganglion neuron degeneration in humans may mean that there will be a longer period for the clinical application of neurotrophic factors to the deafened cochlea.

The combined application of neurotrophic factors and electrical stimulation results in enhanced survival effects on spiral ganglion neurons in the deaf mammalian cochlea in comparison to either treatment alone (Kanzaki et al., 2002; Shepherd et al., 2005). Notably, these anatomical changes have been linked to functional changes in the deafened auditory system, measured by electrically evoked auditory brainstem responses (EABRs). EABR thresholds are significantly higher in deafened unstimulated animals in comparison to normal hearing or electrically stimulated controls (Hardie and Shepherd, 1999). In addition, EABR thresholds continue to rise with increased duration of deafness (Hardie and Shepherd, 1999; Shepherd and Hardie, 2001). The simultaneous delivery of BDNF and CNTF into the cochlea resulted in improved responsiveness of the deafened auditory system, as measured by a decrease in EABR thresholds (Shinohara et al., 2002; Yamagata et al., 2004). Similar reductions in EABR thresholds have been reported following BDNF infusion with and without electrical stimulation (Shepherd et al., 2005), thus illustrating the enhanced physiological and functional benefits of combined therapies. It remains controversial whether delayed treatment with neurotrophic factors can 'rescue' degenerating spiral ganglion neurons after aminoglycoside deafening (Gillespie et al., 2003; Agterberg et al., 2009). These conflicting data suggest that the underlying pathology and extent of neural degeneration play an important role in the potential benefits derived from clinical application of neurotrophic agents in the deaf cochlea. Future research may therefore investigate whether the absolute number surviving ganglion neurons is an important factor in measuring the survival-promoting effect of neurotrophic factor infusion after deafness.

7. Summary

The spiral ganglion neurons of the cochlea represent a defined and isolated population of primary sensory neurons of critical

importance in the normal transmission of sound information to the brain, in both the normal hearing individual and the cochlear implant recipient. A greater knowledge of the anatomical and neurochemical composition of spiral ganglion neurons will aid in the design of potential treatment strategies for their preservation and replacement in the deaf cochlea. Finally, advances in inner ear biology may lead to a better understanding of the complexities of auditory processing in the brain.

Acknowledgments

We are grateful to those researchers who contributed data to this article. We were supported in part by NIH grants DC000232, DC004395, a Life Sciences Research Award from the Office for Medical and Scientific Research, New South Wales, a grant from Advanced Bionics Corporation, the National Health and Medical Research Council of Australia, The University of Melbourne, The Garnett Passe and Rodney Williams Memorial Foundation, and the Royal Victorian Eye and Ear Hospital.

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