Chapter 8
Loss, Degeneration, and Preservation of the Spiral Ganglion Neurons and Their Processes

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Keywords Age-related hearing loss · Animal models · Auditory physiology · Glia · Cell Death · Excitotoxicity · Neural degeneration · Noise-induced hearing loss · Preservation · Primary auditory nerve · Repair · Spiral ganglion · Spontaneous activities

8.1 Introduction

The auditory nerve, consisting of spiral ganglion neurons (SGNs) and their projections, is the primary carrier of auditory information from sensory hair cells of the cochlea to the central auditory system. The loss or dysfunction of SGNs can result in hearing impairment of varying degrees and forms. Loss of SGNs and their processes is frequently reported in the cochlea after hair cell death caused by exposure to noise (Kiang et al., 1976), ototoxic drugs (Bichler et al., 1983; Leake & Hradek, 1988), or genetic deficiency (White et al., 2000). Morphological evidence shows that after intracochlear perfusion with aminoglycosides, SGNs die gradually by the process of apoptosis as indicated by characteristic morphological changes including condensed cytoplasm, nonmarginal clumping of nuclear chromatin, shrinkage and fragmentation of the nucleus and cytoplasm, and the formation of the apoptotic bodies (Dodson, 1997). Pathological identification of SGN degeneration after sensory hair cell loss and the associated molecular mechanisms of neuronal death have been widely discussed (Spoendlin, 1975; Green et al., 2008; Bao & Ohlemiller, 2010). Although it is thought that aminoglycoside damage to SGNs is secondary, recent evidence suggests that there is a primary component of SGN degeneration present after gentamicin exposure (Ruan et al., 2014). This chapter focuses on findings from recent studies of primary and age-related SGN

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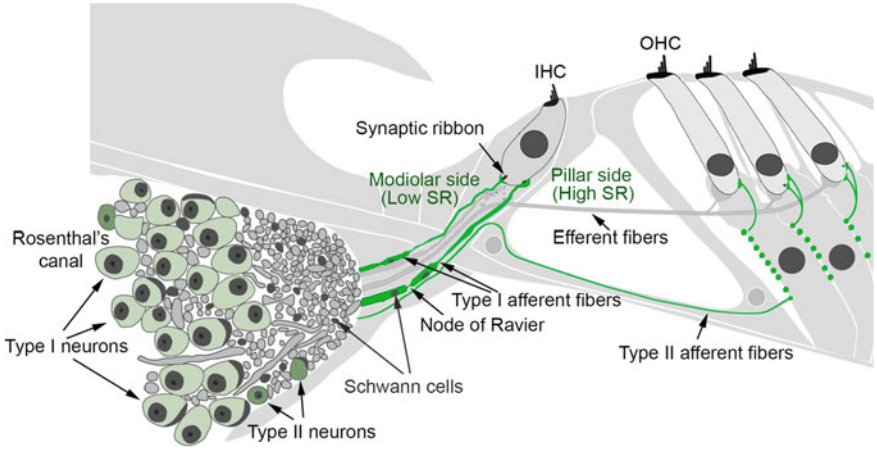

Fig. 8.1 Schematic representation of the relationships between afferent and efferent auditory nerve fibers with two types of sensory hair cells. Spiral ganglion neurons (SGNs) are clustered in Rosenthal's canal (RC) and include two groups of neuronal cells: large type I cells that make up 90–95 % of the SGN population and synapse with inner hair cells (IHCs) and small type II cells comprising 5–10 % of the neurons innervating outer hair cells (OHCs). The cell bodies of type II neurons are often seen in the periphery of RC, toward the osseous spiral lamina. Type I nerve fibers are surrounded by myelinating Schwann cells, whereas type II fibers are enclosed by nonmyelinating Schwann cells. Type I fibers lose their myelin sheath before they enter the organ of Corti through the habenular opening and can be classified into two (or three) populations based on their spontaneous discharge rate (SR). High-SR fibers are thick fibers with large terminals that contact the pillar side of IHCs. In contrast, low-SR fibers are thinner fibers with smaller terminals on the modiolar side of IHCs. Synapses on the modiolar side of IHCs have longer ribbons whereas synapses located on the pillar side of the cell have shorter ribbons. Radial innervations of efferent nerve fibers within the organ of Corti consist of (1) inner spiral fibers that run across the afferent nerve fibers under IHCs and (2) tunnel radial fibers that contact directly to OHC bodies with large nerve endings

degeneration including animal models of neuronal cell degeneration and on potential approaches for preventing auditory nerve degeneration.

The auditory nerve and its associated afferent nerve synapses form part of the structural basis for converting an acoustic signal into an electrical signal and transporting the electrical signal to the central auditory pathway (Fig. 8.1). Briefly stated, sound pressure drives the movement of the basilar membrane to generate a shearing motion of the cilia at the apex of sensory hair cells. This motion results in hair cell depolarization, triggering the exocytosis of neurotransmitter vesicles at afferent synapses located on the basolateral portion of the inner hair cells (IHCs). Neurotransmitters initiate action potentials at the ganglionic initial segments along the auditory nerve. The action potentials then propagate along the nodes of Ranvier within both peripheral and central portions of the auditory nerve carrying sound coding information to higher auditory centers (Robles & Ruggero, 2001; Fuchs et al., 2003; Hossain et al., 2005). Loss or dysfunction in any part of these structures can cause deficits in the conduction of auditory information. Note that the auditory

nerve and its associated elements discussed in this chapter include the eighth nerve extending from hair cell/synapses, dendrites under HCs, SGN cell bodies and central axons within modiolus, and the myelin sheaths provided by Schwann cells, which enclose mostly the peripheral afferent fibers and central axons of the SGNs.

There are two subpopulations of afferent neurons (types I and II) in the spiral ganglia of mammalian cochleas, each with their own morphological, immunostaining, and electrophysiological characteristics (Fig. 8.1; Davis and Crozier, Chap. 4). In most mammalian species (with the exception of humans), the cell bodies of type I SGNs are heavily myelinated. The remaining type II neuronal cells are unmyelinated and innervate the outer hair cells (OHCs) with a 1:10–20 ratio (Kiang et al., 1982; Liberman & Simmons, 1985). Both type I and type II neurons can be found within Rosenthal's canal (RC). Their central projections form the auditory nerve within the internal auditory canal. Closely associated with the peripheral and central processes of these neurons are various glial cells, including Schwann cells, satellite cells, and oligodendrocytes. The peripheral portion of the auditory nerve is surrounded by myelinating Schwann cells (for type I SGNs) and nonmyelinating Schwann cells (for type II SGNs). The central portion of the auditory nerve is also enveloped by Schwann cells in the proximal part (before the glial transition zone) and by oligodendrocytes in the distal part (after the glial transition zone). The central projections of types I and II neurons form the modiolar segment of the auditory nerve, pass through the internal auditory canal and then enter the cochlear nucleus (Nayagam et al., 2011; Muniak et al., Chap. 6).

It is well established that auditory information from the cochlea is redundantly transmitted to the brain through type I SGNs that innervate each IHC (with about 20 synapses per IHC). The functional properties of type II neurons in transmitting auditory information are still largely unknown. Voltage- and current-clamp recordings of SGNs from postnatal rodents revealed rapidly inactivating A-type–like potassium currents in type II neurons (Jagger & Housley, 2003) and slow accommodation of responses to depolarization (Reid et al., 2004). In addition, recordings at the type II synapses show that the release of synaptic vesicles by OHCs results in a small-scale depolarization (Weisz et al., 2009). These results suggest that type II neurons are less active than type I neurons during normal auditory encoding processes. The selective survival of type II neurons has been seen in several cochlear and auditory nerve injury models. For example, pathological alterations of type I SGNs but not type II SGNs were seen after ototoxic lesions of sensory hair cells (Bichler et al., 1983; Leake & Hradek, 1988), noise trauma (Spoendlin, 1975; Lim, 1976), ouabain exposures (Lang et al., 2005), and transection of the cochlear nerve (Spoendlin & Suter, 1976). Future endeavors should address whether unique functional features of type II neurons make them less susceptible to injury in pathological conditions.

Type I afferent fibers are classified into two or three subgroups based on their spontaneous discharge rate and sensitivity to sound stimulation (Fig. 8.1 and Table 8.1). Auditory nerve fibers discharge spontaneously without stimulation.

Table 8.1 Differential physiological and morphological characteristics of high-SR fibers and low- (and medium-SR) fibers

Differentiated characteristics	High-SR fibers	Low- (and medium-) SR fibers
Physiological		
Spontaneous discharge rates		
Cat	18–100 spikes/s	Low-SR: <0.5 spike/s Medium-SR: 0.5–18 spikes/s
Gerbil	18–150 spikes/s	<18 spikes/s for gerbil
Mouse	1–120 spikes/s	<1 spike/s
Response threshold	Low	High
Dynamic range	Smaller	Larger
Threshold recovery following a prior stimulation	Faster	Slower
Sensitivity to endocochlear potential	~1 dB/mV	>1 dB/mV
Morphological		
Peripheral terminal localization	Pillar pole of IHCs	Modiolar pole of IHCs
Peripheral terminal	Larger	Smaller
Ribbon	Shorter and thicker	Longer and thinner
Receptor patch	Larger	Smaller
Synapse vesicle	Less	More
Axon diameter	Larger	Smaller
Mitochondria within terminal	More	Less

The data in the table are based on previous studies in cats (Liberman & Oliver, 1984; Sewell, 1984; Kantardzhieva et al., 2013), gerbils (Schmiedt, 1989; Suryadevara et al., 2001), and mice (Taberner & Liberman, 2005)

Spontaneous rate (SR) in auditory afferent fibers was first examined by Kiang et al. (1965) and then further defined into subgroups by Liberman (1978). In the cat, auditory nerve fibers are classified into three groups: low-SR (<0.5 spikes/s), medium-SR (0.5–18 spikes/s), and high-SR (>18 spikes/s) fibers. Similar SR-based functional subdivisions of auditory nerve fibers have also been reported in other mammalian species including chinchilla (Salvi et al., 1982; Frisina et al., 1996), guinea pig (Winter et al., 1990), gerbil (Schmiedt, 1989; Ohlemiller et al., 1991), and mouse (Taberner & Liberman, 2005). Auditory afferent fibers with higher SRs have low thresholds to stimuli, whereas fibers with lower SRs have higher thresholds (see Table 8.1). In addition, morphological evidence has shown that the specialization of central projections correspond to peripheral fibers based on their SR (review by Nayagam et al., 2011; Muniak et al., Chap. 6).

8.2 Loss of Spiral Ganglion Neurons and Their Processes

Many extrinsic and intrinsic factors can cause the degeneration and dysfunction of SGNs and their processes. These factors include exposure to noise and ototoxic drugs, infection, genetic defects, aging, and absence of auditory signaling input such as loss of sensory hair cells (Liberman & Kiang, 1978; Spoendlin, 1984; Zimmerman et al., 1995). Various loci of pathology in the auditory nerve with a list of representative references are included in Table 8.2. Loss of SGNs and their processes results in auditory impairment by reduction of the auditory information (e.g., timing, neural synchrony, and phase locking) delivered to the brain and by secondary degeneration in cochlear nuclei and other components of the central auditory system.

Table 8.2 A summary on various sites of degeneration reported in SGNs and their associated elements

Anatomic site with pathological changes	General characteristics of nerve dysfunction	Representative references
1. *Synapse*		
Swelling and disruption of postsynaptic structure, reduced synapse ribbons, alterations of synapse location, orphan ribbon	Reduced activity, inexcitability, hyperexcitability (excitotoxicity), reduced activity of low-SR synapses, dys-synchronous auditory procressing, deficits in temporal coding, reduced suprathreshold amplitudes of auditory evoked potential	Liberman and Mulroy (1982), Robertson (1983), Pujol and Puel (1999)
2. *Peripheral process*		
Loss or dysfunction of afferent fibers	Inexcitability, abnormal nerve activity, reduced activity of low-SR fibers, decreased suprathreshold amplitude of auditory evoked potentials	Nadol (1979), Leake and Hradek (1988), Furman et al. (2013)
3. *Neuronal cell body*		
Reduced nuclear area neuronal apoptosis	Inexcitability, no conduction, reduced suprathreshold amplitude of auditory evoked potentials	Leake and Hradek (1988), Dodson (1997)
4. *Central axon*		
Disintegration of myelin sheath, retrograde degeneration of axon	Decreased suprathreshold amplitude, inexcitability, no conduction	Nadol (1979), Webster and Webster (1978)
5. *Myelin sheath*		
Demyelination (axon survives for short period)	Slow nerve excitability, dys-synchronous, slow conduction velocity, long latency response	Leake and Hradek (1988), Jyothi et al. (2010)

It is important to note that ears with signs of SGN degeneration do not always show a significant auditory threshold shift. Previous studies have revealed that cat cochleas with a diffuse loss of about 50 % of auditory nerves still have relatively normal thresholds as measured by behavioral tests (Schuknecht & Woellner, 1955). The amplitude of gross evoked auditory nerve responses depends on a large number of auditory nerves firing synchronously in response to sound. The loss and dysfunction of SGNs and their processes are better identified by auditory suprathreshold measurements, such as the amplitude input/output (I/O) functions of compound action potentials (CAP) (Hellstrom & Schmiedt, 1990; Kujawa & Liberman, 2009). Dysfunction of the auditory nerve can be characterized by threshold elevations, shallow slopes of I/O functions, and diminished maximum amplitudes as compared to healthy ears (see Figs. 8.4 and 8.8).

8.2.1 The Evidence of Secondary SGN Degeneration Following Hair Cell Loss

Degeneration of SGNs can occur as a secondary consequence of cochlear injury. Loss of sensory hair cells leads to a retrograde degeneration and results in a secondary SGN degeneration, a process seen in numerous animal models (see review by Spoendlin, 1984). Direct evidence is still needed to determine the primary or secondary nature of specific neuronal pathological alterations. However, there exists cumulative indirect evidence suggesting that SGN degeneration can occur after hair cell loss, including: (1) temporal patterns: loss of IHCs occurs rapidly after cochlear injury, whereas death of SGNs occurs after hair cell loss (Dupont et al., 1993; McFadden et al., 2004); (2) spatial patterns: the location of SGN loss along the cochlear spiral correlates with the location of hair cell loss (Liberman & Kiang, 1978; Bohne & Harding, 2000); and (3) manipulability of the degeneration: cochlear perfusion of the specific neurotrophic factors that are normally provided by sensory hair cell and/or supporting cells can prolong SGN survival (Ernfors et al., 1996; Altschuler et al., 1999; Stankovic et al., 2004).

Contributing factors to the degeneration of SGN after hair cell loss may include a loss of neural activity and the absence of nerve growth factors, which are critical for neuronal survival (Leake et al., 1999; Fritzsch et al., 1997; Green, 2000). In contrast to a rapid loss of hair cells in many injury models, the secondary degeneration of SGNs is often seen as a slow process with diffuse neuronal cell death. The temporal pattern of SGN death also differs across species; for example, in the rat, a loss of 90 % of the SGNs required approximately 3 months (Bichler et al., 1983). However, in the guinea pig, half of the population of SGNs was still present a year after hair cell loss (Webster & Webster, 1981). In the cat, diffuse neuronal cell loss occurred over several years (Leake & Hradek, 1988). Finally, analysis of human temporal bones suggests that SGNs can survive several decades in human ears devoid of hair cells (Nadol, 1997).

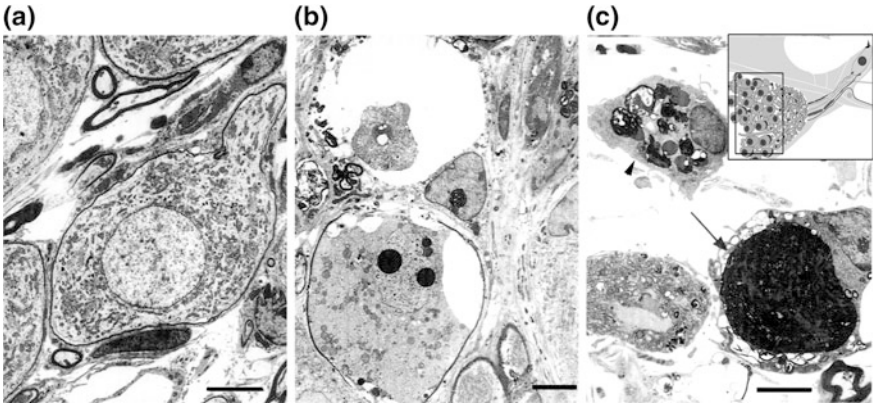

Fig. 8.2 Apoptotic cell death in type I SGNs after cochlear perfusion with aminoglycosides. **a** The ultrastructural features of type I SGNs from the basal turn of a normal guinea pig. **b** A condensed and fragmented nucleus is present in a type I SGN 10 days after aminoglycoside antibiotics were perfused into the perilymph. Separation of organelles is seen in the cytoplasm of the apoptotic neuron with a homogeneous and vesiculated appearance. **c** Another apoptotic neuron (*arrow*) is indicated by the dark chromatin masses. The *arrowhead* points to an activated macrophage identified by the irregular nucleus with clumped chromatin and myelin-associated cellular debris. Scale bars = 5 μm

Similar to other neuronal cells in the nervous system, SGN death after hair cell loss occurs through both necrosis and apoptosis (see review by Hutchins & Barger, 1998); however, apoptosis may be the key mechanism of SGN degeneration in the cochlea. Degeneration of SGNs after hair cell death may occur through two phases. Early-phase cell death occurs as necrosis and/or apoptosis in SGNs following a loss of neural activity due to sensory hair cell loss; the later phase of degeneration results in apoptosis due to pro-apoptotic signaling caused by a chronic stress condition (e.g., loss of neurotrophic support from hair cells and/or supporting cells) (reviews by Fritzsch et al., 2004; Green et al., 2008). Using intracochlear perfusion with aminoglycoside antibiotics, Dodson showed that SGN apoptosis occurred in guinea pig cochleae after hair cell loss (1997). In that study, kanamycin sulfate or gentamicin perfusion led to a rapid loss of hair cells within 3 days and 90 % SGN death within 10 days. Many of these SGNs degenerated through the process of apoptosis, as indicated by characteristic morphological changes including condensed cytoplasm, wrinkling of the nuclear membrane, nonmarginal clumping of nuclear chromatin, and shrinkage and fragmentation of the nucleus and cytoplasm into apoptotic bodies (Fig. 8.2). There is also evidence for necrotic death in SGNs present at an early survival time after kanamycin sulfate or gentamicin perfusion (Dodson, 1997).

The molecular mechanisms of SGN apoptosis have been elucidated mainly through the examination of cultured SGNs with genetic manipulation and pharmacological procedures. These in vitro studies have revealed several pro-survival signaling pathways that are involved in SGN death as a result of the absence of

neural activity or the loss of neurotrophic support (see reviews by Roehm & Hansen, 2005; Green et al., 2008). These signaling pathways include, but are not limited to (1) the cyclic AMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase II and IV systems; (2) pathways involving protein kinase C (PKC), Ca^{2+} signaling, and mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK) activation; and (3) the c-Jun N-terminal kinase (JNK) cell death pathway (Green, 2000; Hansen et al., 2003). In addition, recent in vivo studies have demonstrated that supporting cells in the IHC region and neuregulin–erbB receptor signaling are important for survival of adult SGN (Stankovic et al., 2004; Sugawara et al., 2005, 2007).

8.2.1.1 Primary SGN Degeneration

Animal models of sensorineural hearing loss caused by exposure to noise and ototoxic agents have been established and well-characterized morphologically and functionally for several decades. In many of these models a rapid and robust loss of hair cells was seen before a significant loss of SGNs. However, in a study of aged rat ears, Keithley and Feldman (1982) reported that neuronal degeneration exceeded IHC loss, supporting the hypothesis that neuronal degeneration is not simply retrograde degeneration after loss of IHCs, but is an intrinsic degenerative process. Primary degeneration of SGNs was also seen in aged human cochlea without a robust loss of sensory hair cells (Schuknecht & Gacek, 1993; Makary et al., 2011). In addition, primary neural degeneration was reported in some cases of noise trauma (Spoendlin, 1971; Liberman & Mulroy, 1982), aminoglycoside ototoxicity (Sone et al., 1998), and in the cochleas of white cats with hereditary deafness (Pujol et al., 1977), suggesting SGN degeneration is not a unique secondary event. A series of previous studies have found that degeneration of afferent synapses and progressive loss of SGNs occur in the cochlea when the sensory hair cells are still intact and functional after exposure to an octave-band noise at moderate levels (Kujawa & Liberman, 2006, 2009; Lin et al., 2011; see Fig. 8.4). These data strongly support that SGN degeneration can be independent of the loss of sensory hair cells.

8.2.1.2 Primary SGN Degeneration as a Result of Glutamate Excitotoxicity

Glutamate is the most common excitatory neurotransmitter in the central nervous system and is believed to play an important role in cochlear mechano-neural transduction (Bird et al., 1978; Fuchs et al., 2003). The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor has been identified in type I SGNs (Pujol et al., 1985; Liberman et al., 2011). The excessive release of glutamate results in neuronal damage through excitotoxicity. The pathophysiology of excitotoxicity includes overactivation of

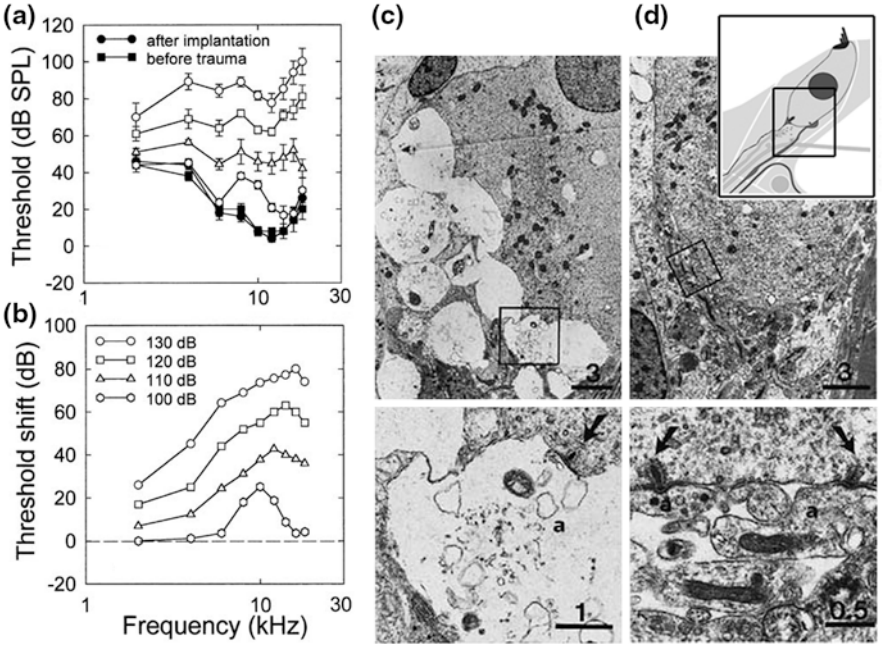

Fig. 8.3 Primary degeneration of afferent dendrites under IHCs after noise exposure in guinea pigs. **a**, **b** Compound action potential (CAP) threshold shifts are present 20 min after different levels of noise exposure (100–130 dB SPL for 15 min). Lesions in the afferent dendrites were present with noise exposures of 120 and 130 dB SPL, but not 100 and 110 dB SPL. **c** Pathological alterations of afferent dendrites are seen under IHCs. The preparation for transmission electron microscopic examination was processed 20 min after 130 dB SPL sound exposure. Massive swellings of afferent dendrites are present under an IHC. *Bottom left panel* enlargement of the area framed in (**c**). A presynaptic ribbon is seen at the basal pole of IHCs adjacent to postsynaptic membrane. **d** Significant protective effect of perilymph perfusion with kynurenate, a glutamate antagonist. No pathologic change of afferent dendrites is observed below an IHC. *Bottom right panel* higher magnification of the area framed in (**d**). An *inset* in the *upper right panel* is a schematic diagram showing the locations of images (**c**, **d**). Scale bar = 0.5 µm (Figure was modified from Puel et al., 1998)

glutamate receptors, influx of high levels of calcium ions (Ca^{2+}) to the postsynaptic cells, and neuronal cell death (Hutchins & Barger, 1998; Martin et al., 1998).

It has been hypothesized that primary SGN degeneration occurs by means of excitotoxic neural damage. Pathological characteristics of primary SGN degeneration include massive swelling of afferent nerve terminals under the basal pole of IHCs and a total disruption of the postsynaptic membrane (Fig. 8.3; Robertson, 1983; Puel et al., 1998). The pathological alterations of afferent dendrites may be caused by excessive presynaptic release of the neurotransmitter glutamate after acoustic stimulation (Eybalin, 1993; Puel et al., 1998; Hakuba et al., 2000). Local application of glutamate agonists can induce pathologic changes in afferent dendrites similar to those induced by noise trauma (Pujol et al., 1985; Zheng et al.,

1997) and the glutamate antagonist kynurenate can protect SGN dendrites from acoustic damage (Puel et al., 1998). Afferent terminals can fully or partially recover from excitotoxic damage and this recovery may play a role in the phenomenon of temporary threshold shift (TTS) (Liberman & Mulroy, 1982; Robertson, 1983; Puel et al., 1996). However, loss of presysnaptic ribbons and progressive SGN loss after TTS has also been reported in recent studies (Kujawa & Liberman, 2006, 2009; Fig. 8.4).

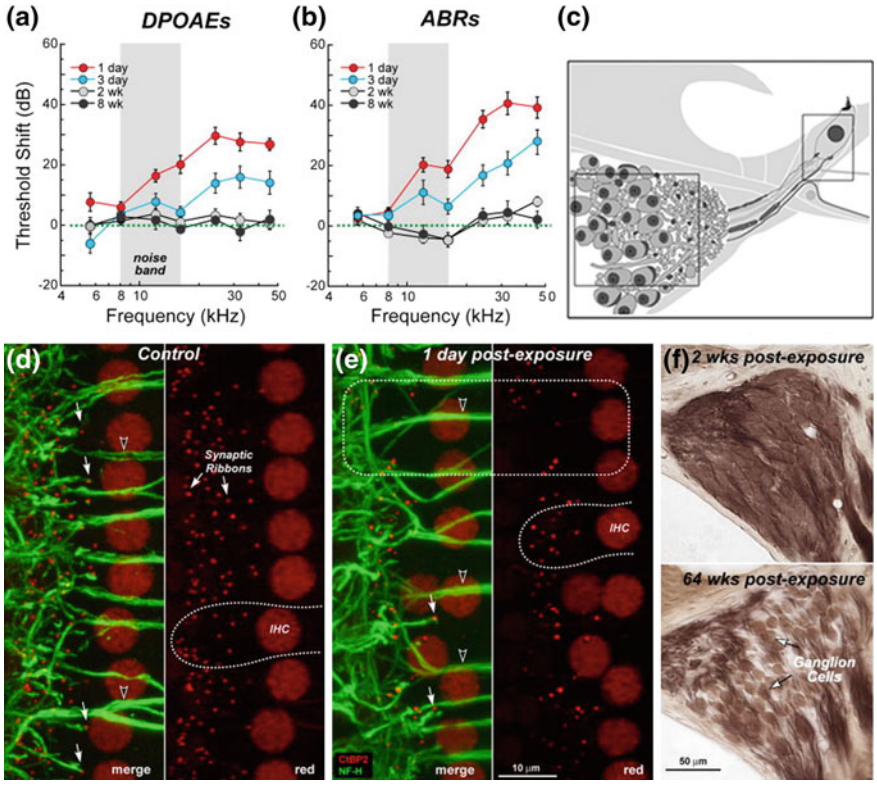

Fig. 8.4 Primary SGN degeneration following noise-induced temporal threshold shifts. **a**, **b** Temporal threshold shifts are seen in the measures of ABRs and DPOAEs in mice after exposure to an 8–16 kHz octave-band noise at 100 dB SPL for 2 h. **c** Schematic representation of the locations of the SGNs and the afferent terminals under IHCs examined in the studies. **d**, **e** A rapid and robust loss of afferent synaptic ribbons (anti-CtBP2, *red*; *arrows*) in the flat preparations of cochleae at the 32 kHz region occurred 1 day after noise exposure. Auditory nerve and afferent dendrites were stained with anti-heavy neurofilament antibody (*green*, *arrowheads*). *White dashed lines* indicate the outlines of IHCs in the control (**d**) and noise-exposed (**e**) ears. A *dashed box* in (**e**) shows the region with significant reduction of both $CtBP2^{+}$ presynaptic ribbons and $neurofilament^{+}$ postsynaptic terminals. Note that the anti- CtBP2 antibody also stains IHC nuclei and anti-neurofilament also stains efferent processes to OHCs. **f** Cross sections show a diffuse SGN loss occurring 64 weeks after noise exposure in the 32-kHz regions of the cochlea (Figure was modified from Kujawa & Liberman, 2009)

8.2.1.3 Primary SGN Degeneration After Noise Exposure

Primary degeneration of SGNs after noise exposure has been understood largely based on experiments associated with glutamate receptor antagonists. However, questions remain on several critical issues. First, high-level noise exposures (e.g., 130 dB SPL pure tone used by Puel et al., 1998) were often applied to generate at least two types of cochlear lesions: (1) loss of hair cells starting with OHCs at lower levels then including IHCs at higher levels; and (2) a massive destruction of afferent terminals below IHCs as shown in Fig. 8.3. Although pathological nerve alterations were seen as early as 20 min after sound exposure (suggesting this pathology is independent of hair cell loss), direct evidence of primary degeneration is still needed from a model with only auditory nerve injury. Also, an evaluation of whether excitotoxic lesions in the afferent nerve terminals are able to recover fully and whether auditory nerves regenerate after noise trauma is still needed. Until recently, most of the morphological observations in these studies were performed at the ultrastructural level. Longitudinal evaluations of dynamic changes in afferent synapses and afferent nerve terminals under IHCs are extremely challenging, and quantitative analysis of morphological alterations in the auditory nerve is virtually absent from these earlier studies. Finally, because lesions are mixed in these models, comprising losses of afferent synapses, SGNs, IHCs, and OHCs, it is difficult to determine which component contributes to the various noise-induced auditory functional deficits.

Recently, newly developed genetic, biochemical, electrophysiological, and high-resolution optical approaches have provided tools for the quantitative examination of the degeneration of SGNs and their associated elements (Khimich et al., 2005; Weisz et al., 2014; Rutherford and Moser, Chap. 5). Numerous biological markers for synapses and nerve terminals have been identified and characterized, including antibodies for the presynaptic ribbon (RIBEYE/transcription factor CtBP2; Khimich et al., 2005), postsynaptic glutamate receptor patches (GluR2/3; Matsubara et al., 1996), unmyelinated nerve terminals (neurofilament; Berglund & Ryugo, 1991), and afferent terminal swellings (parvalbumin; Kujawa & Liberman, 2009). A series of studies using (1) high-powered confocal imaging of sensory epithelium, (2) three-dimensional quantification of ribbon synapse numbers, and (3) histological quantification of the neuronal cells demonstrated that a moderate level of noise exposure can cause a permanent loss of afferent synapses without hair cell damage (Kujawa & Liberman, 2009; Lin et al., 2011; Furman et al., 2013). These studies showed a rapid and selective loss of afferent synaptic ribbons under IHCs after progressive degeneration of SGNs occurring in mice after exposure to an 8–16-kHz octave-band noise at 100 dB SPL for 2 h (Fig. 8.4). Shortly after noise exposure, auditory brain stem responses (ABRs) were elevated about 40 dB concurrent with a slightly smaller threshold elevation of distortion product otoacoustic emissions (DPOAEs)—a measure of OHC function. By 2 weeks after noise exposure, ABR and DPOAE thresholds were back to normal, preexposure levels. Even though cochlear threshold sensitivity fully recovered, the ABR wave I amplitudes were reduced significantly at high stimulus levels at frequencies strongly affected by the noise. Together, these groundbreaking studies provide

direct evidence that primary degeneration can occur in the inner ear in response to pathological stress conditions.

8.2.1.4 Primary SGN Degeneration and Dysfunction Associated with Gene Defects

Genetic studies of sensorineural hearing loss have progressed at a rapid pace in recent years. To date, more than 64 genes and 125 loci that link to various degrees of hearing impairment have been identified (reviews by Dror & Avraham, 2010; Angeli et al., 2012). Some of these genes play important roles in the regulation of synaptic transmission and neuronal survival and death. Deficiency of these genes likely contributes to primary SGN degeneration. Santarelli (2010) reviewed the genes associated with human auditory neuropathy, diagnostically characterized as having abnormal ABRs and completely preserved otoacoustic emissions (OAEs). Here, a brief review was given on several well-documented genes that are associated with auditory neuropathy—*SLC17A8*, *OTOF*, *PJVK*, and *DIAPH3*. In addition, animal studies of gene defects revealed two transcription factors, nuclear factor κB (NF-κB) and forkhead box O3 (FoxO3), that play important roles in maintaining the survival of SGNs and normal function of the auditory nerve and the IHC synapse (Lang et al., 2006; Gilels et al., 2013).

Vesicular glutamate transporter VGLUT3 (SLC17A8, DFNA25) and otoferlin (OTOF, DFNB9) are two key components of the afferent synapse on IHCs. VGLUT3, one of the three subtypes of vesicular glutamate transporters (VGLUT 1-3), is selectively expressed in IHCs and responsible for loading the synaptic vesicles with glutamate (Ruel et al., 2008; Seal et al., 2008). Mice lacking VGLUT3 have no auditory brainstem responses but have robust OAEs, indicating an appearance of normal OHC function. A significant reduction of IHC synapse numbers and pathological alterations of SGNs were also observed in these mice. Otoferlin is a multi-C_2 domain protein essential to the exocytosis of synaptic vesicles in IHCs and the consequent action of the Ca^{2+} sensor triggering membrane fusion at the IHC ribbon synapse (Yasunaga et al., 1999; Roux et al., 2006). Otoferlin-deficient mice ($Otof^{-/-}$) lacking exons 14 and 15, which encode most of C_2C domain, are totally deaf but have preserved OAEs. Although normal IHC ribbon synapses were observed in postnatal $Otof^{-/-}$ mice, the pathological alterations of SGNs have not been determined (Roux et al., 2006).

Pejvakin, encoded by PJVK, is a 352-residue protein belonging to the gasdermin protein family and is expressed in cochlear hair cells, supporting cells, and SGNs (Delmaghani et al., 2006). Abnormal expression of this protein is associated with nonsyndromic auditory neuropathy DFN59 and also DFNA5, which participates in the p53-regulated cellular response to DNA damage (Masuda et al., 2006). Mice lacking pejvakin ($Dfnb59t^{m1Ugds}$) show an elevation of ABR thresholds but normal OAEs at affected frequencies. Examination of the organ of Corti via scanning electron microscope revealed no structural abnormalities, but a detailed examination of auditory nerve morphology was not included. The Diaphanous homolog 3

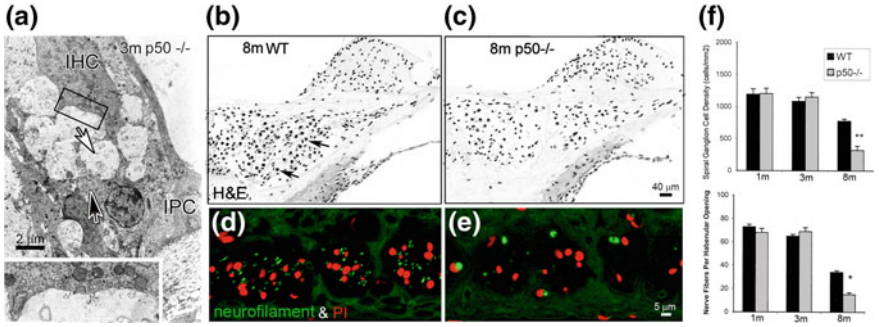

Fig. 8.5 Primary degeneration of the auditory nerve in nuclear factor-κB deficient mice. **a** Ultrastructural features of the basal half of an IHC and its subcellular synaptic region from the basal turn of a 3-month-old $p50^{-/-}$ mouse. Membranous structures presumably representing residue from degenerated cell organelles were seen in the $p50^{-/-}$ mice. Numerous small vesicles infiltrated with mitochondria and short profiles of cisternae appear in the cytoplasm in the base of the IHC. An inner pillar cell (IPC) and border cell enclosed the IHC and nerves consisting of intermingled afferent inner radial fibers (*white arrow*) and efferent spiral fibers (*black arrow*). The efferent inner spiral fibers and terminals (*white arrow*) appear normal. **b–f** Cross sections of the spiral ganglia in the basal turn of an 8-month-old wild type (WT) (B) and $p50^{-/-}$ mouse (**c**). **g** SGN counts in the basal turn in 1-, 3-, and 8-month-old WT and $p50^{-/-}$ mice. The density of SGNs in the 8-month-old $p50^{-/-}$ mice was about half that of the WT controls and the difference was significant (ANOVA, $p < 0.01$) (Figure was replotted from Lang et al., 2006)

(*DIAPH3*), which encodes the diaphanous-3 protein, was mapped to the autosomal dominant auditory neuropathy, dominant 1 (AUNA1). Analysis of lymphoblastoid cells showed an upregulation of *DIAPH3* mRNA expression suggesting a gain of function effect present in AUNA1-affected patients (Schoen et al., 2010). Expression of a constitutively active form of the diaphanous protein in *Drosophila* leads to a deficiency of auditory response from the auditory organ.

The transcription factor NF-κB has a fundamental role in regulating inflammatory responses and apoptosis in response to injury in many cell types (Barkett & Gilmore, 1999). The p50/p65 heterodimer is the predominant complex of NF κB in most mammalian cells. NF-κB is expressed and shows a low-level constitutive activity in the neurons of the central nervous system (Kaltschmidt et al., 1994). By using measures of cochlear function and histopathological evaluation, an accelerated hearing loss with correlated primary degeneration of SGNs and afferent nerve processes was seen in the SGNs of $p50^{-/-}$ mice (Lang et al., 2006). As shown in Fig. 8.5, marked excitotoxic-like alterations were seen at afferent terminals under IHCs of young adult $p50^{-/-}$ mice (1–3 month old). In contrast, no major pathological changes were seen in OHCs or the stria vascularis in the same cochleas. At 8 months of age, the density of SGNs in the basal turn of the knockouts was only about half that of wild-type mice. However, neither significant EP loss with age nor accelerated degeneration of hair cells was seen in the same cochleas, indicating that the loss of SGNs and auditory nerves is primary and independent of the degeneration of sensory hair cells.

FoxO3 is a transcription factor belonging to the forkhead O subclass, which is characterized by a distinct forkhead DNA-binding domain. It plays an important role in the regulation of stress response proteins in a variety of pathological conditions, including excitotoxic damage in brain tissue (Brunet et al., 1999; Davila et al., 2012). Adult mice lacking FoxO3 have elevated ABR thresholds but normal OHC function. Comprehensive histological examinations of cochlear tissues revealed that alterations of synapse locations and degeneration of the afferent nerve cause age-related hearing loss in these mice (Gilels et al., 2013).

8.3 Age-Related SGN Dysfunction and Degeneration

Age-related hearing impairment affects about 40 % of adults older than 65 years of age (Gates & Mills, 2005; Gordon-Salant & Frisina, 2010). By the age of 80, about half of the population suffers from varying degrees of hearing loss and/or has difficulty understanding speech under difficult listening conditions. The most widely accepted pathological categories of age-related hearing loss were established by Schuknecht and Gacek (1993). Four types of human presbycusis were initially described: (1) sensory, mainly affecting the sensory hair cells; (2) neural, typified by the loss of SGNs; (3) metabolic, characterized by atrophy of the stria vascularis; and (4) mechanical, featuring a stiffened basilar membrane and organ of Corti. After additional research on human temporal bones, Schuknecht and Gacek (1993) described neuronal loss as the most persistent and the best indicator of age-related degeneration in the cochlea. In contrast, sensory cell loss is thought to be the least important cause of hearing loss in older adults. The primary SGN degeneration has been consistently seen in a variety of inner ear disorders including Ménière's disease (Nadol & Thornton, 1987), sudden deafness (Ishii & Toriyama, 1977; Schuknecht & Donovan, 1986), Usher's syndrome (Nadol 1988), and Friedreich's ataxia (Spoendlin, 1974). Otte et al. (1978) examined 100 human temporal bones from donors in whom there was no evidence of diseases affecting the cochlea. Loss of SGNs occurred at a rate of about 2100 neurons per decade. In a more recent human temporal bone study, quantitative analysis of another 100 human temporal bones from donors, ranging in age from newborn to 100 years old, revealed a uniform progressive SGN loss at a mean rate of 100 cells per year. The human temporal bones examined in this study separated cases with and without significant cochlear hair cell loss (Makary et al., 2011; Fig. 8.6).

Degeneration and dysfunction of SGNs and their processes are also commonly seen in other types of age-related hearing loss described by Schuknecht, including hearing loss exhibiting sensory and metabolic (strial) characteristics. Damage to various cochlear components can lead to abnormal activity of the auditory nerve. Specifically, elevation of auditory nerve thresholds or changes of auditory nerve tuning curves can result from any of the following: total or partial loss of IHCs or OHCs (Kiang et al., 1976; Schmiedt & Zwislocki, 1980; Kiang et al., 1986), damage to stereocilia on IHCs or OHCs (Liberman & Kiang, 1984), and

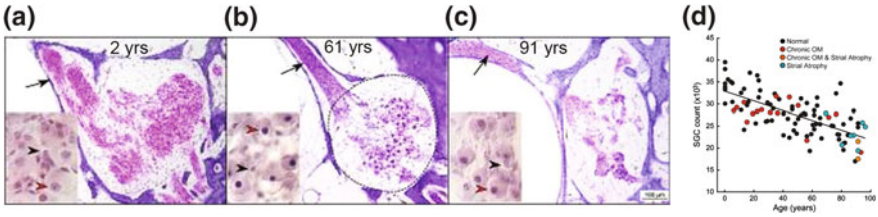

Fig. 8.6 Loss of SGNs in human cochleae. Horizontal sections of human spiral ganglions, which were obtained from donors aged 2 (**a**), 61 (**b**), and 91 (**c**) years, were stained with hematoxylin-eosin. *Black arrows* point to the peripheral portion of the auditory nerve within the osseous spiral lamina. The *dashed line* in (**b**) gives the approximate outline of Rosenthal's canal (RC). High-powered images of some cells within RC are present in the small *inset* in each panel. SGNs with a diffuse cytoplasm are indicated with *red arrowheads*. The *black arrowheads* indicate glial cell nuclei. **d** SGN counts from 100 human cochlea indicate an age-dependent loss of neuronal cells from a mean of 33,679 in the first decade to 22,444 in the tenth decade. Among the 100 ears in the studies, none had significant loss of sensory hair cells, 75 had no significant pathological changes in middle and inner ear (*black solid circles*), 7 had strial atrophy (*blue solid circles*), 16 had chronic otitis media (OM) (*red solid circles*), and two had both OM and strial atrophy (*orange solid circles*). The best-fit straight line is plotted (*solid line*) (Figure was modified from Makary et al., 2011)

degeneration or loss of the cells in the stria vascularis and spiral ligament (Kiang et al., 1986; Lang et al., 2010; Schmiedt, 2010). Comprehensive analyses with multiple functional tests, including auditory threshold and suprathreshold measurements such as CAP or ABR wave I amplitudes, DPOAEs, and endocochlear potential (EP) measurements, are necessary for discriminating SGN loss from other cochlear lesions in the aged ears.

8.3.1 *Age-Related SGN Dysfunction: Selective Loss of Low-SR Fiber Activity*

Primary afferent auditory nerves can be classified into two or three groups based on their SR and response threshold (Fig. 8.1 and Table 8.1). Low-SR fibers often have substantially higher thresholds and wider dynamic ranges in response to sound (Liberman & Kiang, 1978; Schalk & Sachs, 1980; Liberman, 1982) and are more resistant to masking in the presence of continuous broadband noise. Lower thresholds and smaller dynamic ranges make high-SR fibers saturate more rapidly in response to loud sounds. Consequently, low-SR fibers respond best to higher level sounds and in environments with continuous background noise (Costalupes et al., 1984; Young & Barta, 1986). Low-SR fibers at high levels preserve auditory information, especially for coding sound intensity, timing and spatial information, and amplitude modulation (Zeng et al., 1991; Frisina et al., 1996). Morphological evidence has shown that spatial segregation of central projections related to SR appears in the cochlear nucleus. Low-SR fibers give rise to larger axon arborization

and reach a wider range of regions within the cochlear nuclei than high-SR fibers (Fekete et al., 1984; Liberman, 1993; Ryugo, 2008). In addition, the central components of low-SR auditory fibers project to the areas where the neurons of the medial olivocochlear efferent system are located (Ye et al., 2000), suggesting that low-SR fibers are associated with a high threshold feedback system in the inner ear, in particular for processing loud stimuli. Moreover, human speech processing is conducted in the intensity regions (between 40 and 90 dB SPL) where the low-SR fibers become active, even while the high-SR fibers are saturated. Together, substantial evidence suggests that low-SR fibers are critical in environments that involve complicated auditory processing and speech understanding under difficult listening conditions. Thus, the age-related loss or inactivity of low-SR fibers may play a role in the decline of speech understanding in older adults (Dubno et al., 1984; Halling & Humes, 2000).

Recordings from single auditory nerve fibers can provide direct insight into the functional state of SGNs and their processes (Kiang et al., 1965). Previous studies using a well-established gerbil model of age-related hearing loss indicated reduced activity of the low-SR fiber population (Schmiedt, 1989; Schmiedt et al., 1996). These animals were raised in a sound-conditioned vivarium where the average sound level was 40 dBA. Scattered OHC loss was seen in the apical and basal turns of the aged gerbils with little or no IHC loss. The major pathological alteration in these animals was the degeneration of SGNs and cells in the cochlear lateral wall, which contributes to the generation and maintenance of the EP. Physiological characterization of single auditory-nerve fibers revealed that the percentage of low-SR fibers with characteristic frequencies (CF) greater than 6 kHz decreased from approximately 57 % in young controls to approximately 29 % in quiet-aged gerbils (Fig. 8.7), suggesting that low–SR fibers with CF greater than 6 kHz either degenerate or become inactive with increased age. To validate this result, a method for population studies of auditory nerve activity developed by Relkin et al. (1995) was also applied. This approach measures the recovery of the CAP amplitude after prior stimulation with a probe tone. CAP recovery curves include two segments: a fast segment, reflective of quick recovery of high-SR fibers, and a slow segment associated with the activities of low-SR fibers. CAP recovery curves from aged gerbils revealed a faster recovery than young controls at 8 and 16 kHz, indicating a loss of low-SR fiber activity in the aged animals.

8.3.2 Selective Loss of Low-SR Fibers as a Result of EP Reduction

In addition to the loss and/or shrinkage of the SGNs and radial fiber population seen in quiet-aged gerbils (and other aged rodents including rats and mice) compared to young controls (Keithley et al., 1989; White et al., 2000), a significant degeneration

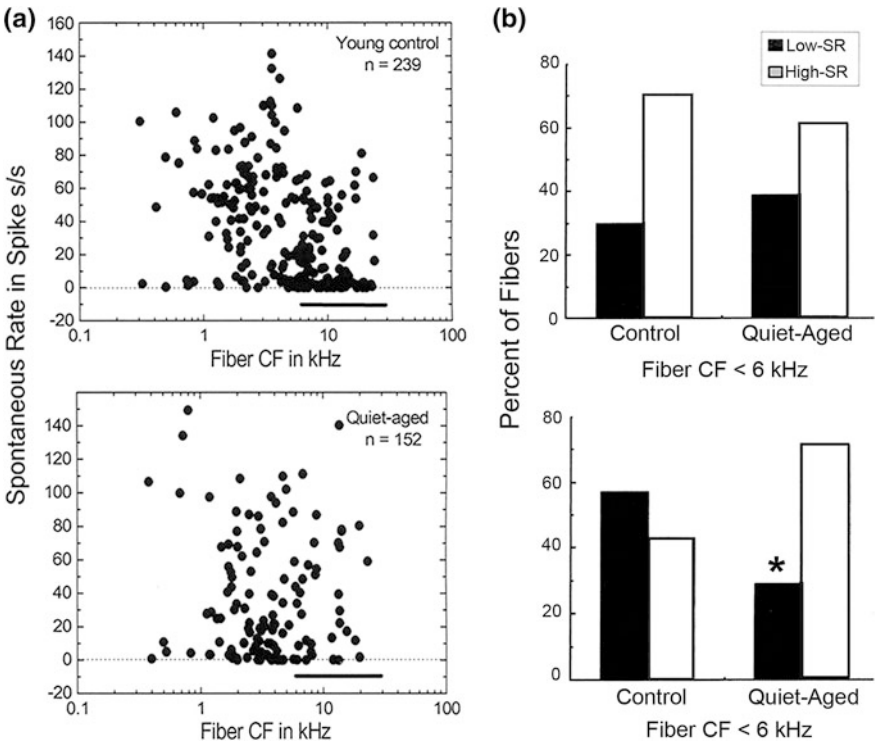

Fig. 8.7 Decline of low-SR fiber activity in quiet-aged gerbils. **a** Distribution of fiber SR plotted as a function of nerve fiber CF shows a reduction of low-SR fiber population for CFs > 6 kHz in quiet-aged gerbils as compared to young controls. **b** Percentages of low-SR fibers with CFs > 6 kHz are significantly different between quiet-aged gerbils and young controls (Figure was replotted from Schmiedt et al., 1996)

of the cochlear lateral wall is also seen in most of these older animals (Schulte & Schmiedt, 1992; Hequembourg & Liberman, 2001; Mills et al., 2006). Can a chronic EP reduction as a result of age-related degeneration of the cochlear lateral wall have a negative impact on auditory nerve activity, particularly on the activity of low-SR fibers? To test this hypothesis, the activity of single auditory nerves were recorded from a young animal model of age-related hearing loss, which was established by the chronic application of furosemide to young adult gerbils (Schmiedt et al., 2002). Application of furosemide to the round window of young animals can cause the chronic reduction of EP as seen in older animals. In this model, the only significant pathological alteration is limited to the hook region of the cochlear lateral wall including a loss of strial intermediate cells and edema in the stria vascularis, whereas the morphology of SGNs and sensory hair cells appear normal (Lang et al., 2010). Reduction of EP levels and alterations of suprathreshold neural responses in furosemide-treated animals resemble that of quiet-aged gerbils

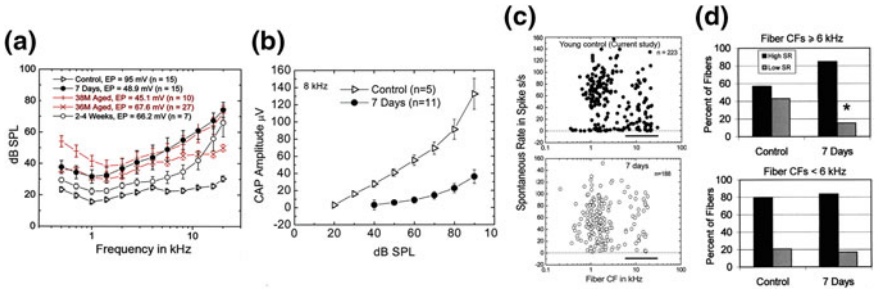

Fig. 8.8 The decline of the low-SR activity as a result EP reduction. **a** Chronic EP reduction results in CAP thresholds in the furosemide-treated ears that resemble those in quiet-aged ears. Average CAP thresholds and EPs values in gerbils treated with furosemide for 7 days are similar to the alterations of CAP thresholds and EPs values of two groups of quiet-aged gerbils (Schmiedt et al., 2002). **b** CAP amplitude input/output (I/O) functions identified the alterations of suprathreshold neural responses in the furosemide-treated ears. The flattened slope of the CAP I/O function obtained in the furosemide-treated ears indicates a decline in the evoked auditory nerve activity in those animals. **c**, **d** Single-unit recording from the auditory nerve shows alterations of SRs across CF in furosemide-treated and control animals. The percentages of low-SR fibers were significantly different in young compared to furosemide-treated animals ($p < 0.01$, chi-square test), similar to that in quiet-aged animals (Schmiedt et al., 2002). (Images were modified from Lang et al., 2010)

(Fig. 8.8a, b). Quantitative evaluation of the spontaneous activity of single auditory fibers in these animals revealed a loss of the low-SR fiber population (Fig. 8.8c, d). CAP recovery functions also indicated a decline in activity of the low-SR fibers with CFs greater than 6 kHz in the same animal model (Lang et al., 2010). These results support the hypothesis that age-related dysfunction of the auditory nerve can be a direct result of the degeneration and/or dysfunction of the cochlear lateral wall and the subsequent chronic reduction of EP levels.

8.3.3 Noise-Induced Primary SGN Degeneration in Aged Ears and Selective Loss of Low-SR Fibers After Noise Exposure

It has been generally accepted that exposing the ear to noise over a lifetime is likely to have a significant impact on the processes of normal age-related hearing loss. A longitudinal clinical study revealed that noise-induced hearing loss (NIHL) occurring at a young age accelerates the hearing loss at a frequencies adjacent to the noise bandwidth (Gates et al., 2000). However, the underlying mechanisms and interaction between NIHL and age-related hearing loss are difficult to determine in these clinical studies (Mills et al., 1998; Lee et al., 2005; Gates, 2006). Recent animal studies using mild levels of octave band noise (that result in a temporary

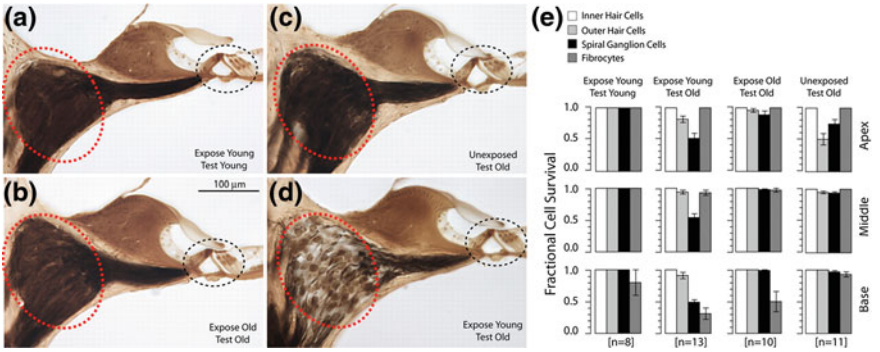

Fig. 8.9 Early noise exposure accelerates primary SGN degeneration in aged ears. Exposure of young (**a**, **d**) and old (**b**) mice with 8–16 kHz octave-band noise at 100 dB SPL for 2 h. A significant loss of SGNs with intact sensory hair cells is seen in old mice that receive noise exposure at a young age (**d**), but not in young mice that were recently exposed to noise (**a**), or in old mice that received (**b**) or did not received (**c**) noise exposure at an old age. *Large and small circles* point to SGNs and the organs of Corti, respectively. Semiquantitative analysis of IHCs, OHCs, SGNs, and spiral ligament fibrocytes shown in (**e**) are present for "Exposure Young Test Young" (exposed at 5.5 weeks and tested at 7.5 weeks), "Exposure Young Test Old" (exposed at 5 weeks and tested at 100 weeks), "Exposure Old Test Old" (exposed at 124 weeks and tested at 126 weeks), and "Unexposed Test Old" (tested at 105 weeks) animals (Figure was modified from Kujawa & Liberman, 2006)

threshold shift in mice and guinea pigs) have demonstrated that noise exposure can cause a rapid loss of nerve terminals and delayed primary SGN degeneration while leaving sensory hair cells intact. Further, early noise exposure results in a significant loss of afferent synapses, accelerated primary SGN degeneration and early onset of age-related hearing loss (Fig. 8.9; Kujawa & Liberman, 2006, 2009; Sergeyenko et al., 2013). A mild level of noise exposure also can cause a significant loss of low- and medium-SR fibers, indicated by single unit auditory nerve recordings (Furman et al., 2013). These findings provide direct evidence that age-related SGN degeneration can be a primary event following mild levels of noise exposure without a significant permanent threshold shift. Thus, noise exposure can cause loss and/or inactivation of low- and medium-SR fibers similar to that seen in aged gerbil ears (Schmiedt, 1989; Schmiedt et al., 1996).

The finding of loss and/or dysfunction of low-SR fibers in the aged cochlea with chronic EP reductions, as well as in young adult cochleas after noise exposure in animal models have broadened our understanding of the causes of the hearing deficits seen in older adults. Yet, many questions remain. For example, is the loss of low-SR fiber activity a result of degeneration of the low-SR fibers or have the characteristics of the low-SR fibers been altered? What mechanism could cause alterations in the SR of the auditory nerve with aging and noise exposure? How does the loss of afferent synapses contribute to the dysfunction of low-SR fibers?

8.4 Animal Models of Primary Spiral Ganglion Neuron Degeneration

Degeneration of SGNs accompanied by or in the absence of sensory hair cells is generally not a rapid process. The process of neuronal death is slow; in fact, it took months or years to see a significant reduction in neuron counts in injured cochleas by partially sectioning the auditory nerve through the posterior cranial fossa (Schuknecht & Woellner, 1955) or after exposure to noise or ototoxic drugs (Leake & Hradek, 1988; Kujawa & Liberman, 2006). This slow pace of neuronal degeneration makes it challenging to obtain enough protein or RNA samples associated with SGN death to investigate the underlying mechanisms of SGN degeneration using currently available cellular and molecular biological assays.

8.4.1 Primary SGN Degeneration After Ouabain Exposure

Ouabain, also known as g-strophanthin, is a cardiac glycoside that specifically binds to the plasma membrane of Na^+, K^+-ATPase and blocks its activity at higher concentrations (Hernández, 1992). Application of ouabain to the intact round window (RW) membrane of adult gerbil cochleas via acute or chronic infusion induces a rapid apoptotic death of about 90 % of the SGNs within 12–24 h. This treatment has a minimal effect on sensory hair cells and other cochlear cell functions (Schmiedt et al., 2002; Lang et al., 2005). Like that observed in adult gerbils, ouabain treatment of adult CBA/CaJ mice also causes substantially elevated neural thresholds and a loss of the majority of SGNs within a few days, whereas other cochlear cells appear intact functionally and morphologically (Fig. 8.10). Given anatomical differences of the round window niche between mouse and gerbil, the procedure used in mice has been modified by employing repeated application of a fresh solution of 1 mM ouabain to the round window at 10–15-min intervals over a 45–60-min duration (Lang et al., 2011; Yuan et al., 2014). This method has been successfully applied in several other mouse strains, revealing similar patterns of SGN degeneration.

8.4.2 Selective Loss of Type I SGNs in Ouabain-Treated Ears

In the ouabain model, apoptotic death occurs in type I SGN neurons, but not in type II SGNs (Lang et al., 2005, 2011; Fig. 8.11). Quantitative immunohistochemical analysis of ouabain-treated ears in both mice and gerbils revealed that surviving neurons (about 7 % of total SGNs) are stained positively with peripherin, a marker

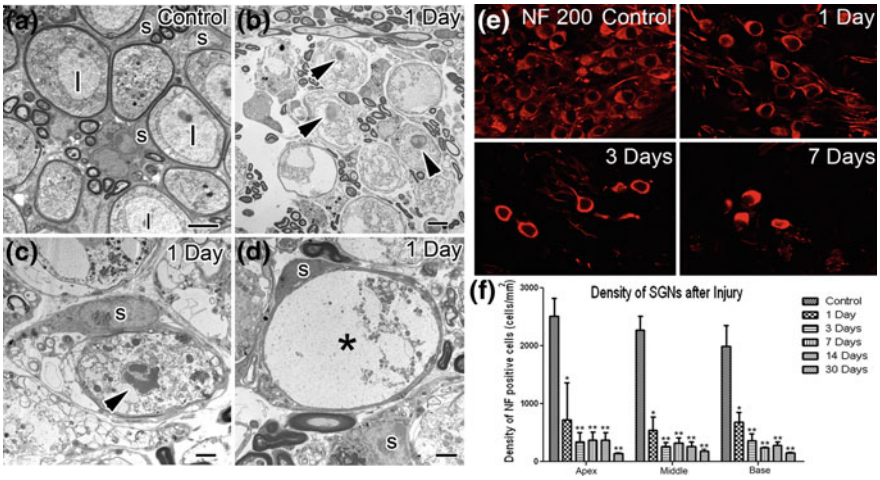

Fig. 8.10 Rapid type I SGN degeneration in ouabain-exposed mice. **a** Type I (I) SGNs are enclosed by glial cells (s) in a normal ear. **b**, **c** Apoptotic-like cell death (*arrowhead*) appears one day after ouabain exposure. **d** Surviving glial cells (s) enclose the debris of the degenerative SGN (*asterisk*). **e** SGNs were stained positively for neurofilament 200 (NF 200, a neural marker, *red*) in a young adult mouse. Many NF^+ cells are missing at 1, 3, and 7 days post-exposure. **f** Cell counts show significant losses in the mean densities of NF^+ SGNs in the apical, middle and basal portions of the cochleas from control, and 1, 3, 7, 14, and 30 days after ouabain exposure. Scale bars, 5 μm in (**a**, **b**); 2 μm in (**c**, **d**) (Figure was modified from Lang et al., 2011)

for type II neurons. The morphological and immunocytochemical features of type II neurons appeared normal even 1 month after ouabian exposure. The release of cytochrome *c*, poly (ADP-ribose) polymerase (PARP) cleavage, and activation of caspase 3 are three crucial events that initiate the processes of apoptosis (Fiskum, 2000; Ha & Snyder, 2000; Chiarugi & Moskowitz, 2002). Evidence from immunostaining shows that cytochrome c redistribution, PARP degradation, and caspase 3 activation occur in type I, but not in type II neurons (Lang et al., 2005). Calcineurin is another important signaling molecule that is involved in modulating neuronal cell survival in response to extracelluar stress (Morioka et al., 1997; Lilienbaum & Israël, 2003). The high level expression of calcineurin protein in type II but not in type I neurons may be associated with the selective survival advantage of the type II neurons (Lang et al., 2005). Ouabain-treated animals express a selective loss of type I SGNs with little effect on sensory hair cells and the cochlear lateral wall. Thus, the ouabain model is a gold standard for the study of cellular and molecular mechanisms of type I SGN degeneration. The ouabain model can also be used to (1) study how central auditory neural circuits reorganize themselves in response to the pathophysiological situation of having peripheral input from type II afferent neurons only; (2) characterize the electrophysiological and biophysical features of the type II neurons; (3) determine whether the loss of type I afferent neural activity generates central hyperacusis or tinnitus; (4) evaluate the self-repair

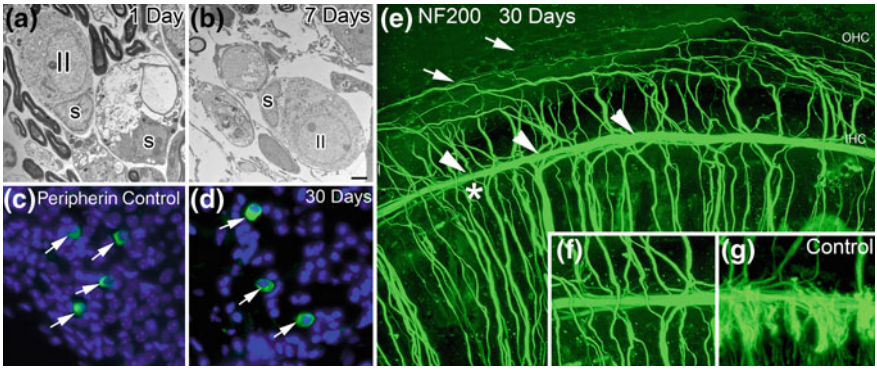

Fig. 8.11 Selective degeneration of type I SGNs in ouabain-treated mice. **a** A type II SGN with nonmyelinating glial cell (s) is present in the apical turn of a mouse 1 day after ouabain exposure. **b** Another type II SGN enclosed by a nonmyelinating glial cell (s) is present in the middle turn of a mouse 7 days post-treatment. **c**, **d** Peripherin^{+} type II SGNs (*green*, *arrows*) in the apical turns of control and 30 day post-treated ears. Nuclei were counterstained with bisbenzimide (*blue*). **e** Surface preparation from the apical turns of a mouse 30 days after treatment shows loss of NF 200^{+} afferent fibers (*arrowheads*) innervating IHCs in the apical turn of a mouse at 30 days post-treatment. **f** An *inset* shows an enlarged image in (**e**) in the area underlying IHCs (*asterisk*) compared to a similar location in a control ear (insert **g**). The absence of radial afferent fibers allows a clear view of the inner spiral bundle including both efferent and afferent fibers projecting into the outer hair cell (OHC) region. Numerous external spiral fibers are seen tracking spirally into the OHC region including long thin type II afferent fibers (*arrows*). Scale bar = 15 μm in (**a**) and (**b**) (Figure was modified from Lang et al., 2011)

potential of cells in the auditory nerve after acute injury; and (5) examine how conditions of cochlear nerve injury affect the outcome of stem cell transplantation or genetic manipulation.

8.4.3 Primary SGN Loss Induced by Central Process Lesion

A partial sectioning of the auditory nerve was first used for the treatment of Ménière's disease (Green & Douglass, 1951). In an animal study of SGN activity, Schuknecht and Woellner (1955) performed a partial section of the auditory nerve within the internal auditory meatus in cats. This surgical procedure induced a diffuse loss of SGNs to varying degrees. Primary SGN degeneration can also be induced by compression given at the internal auditory meatus in rats (Sekiya et al., 2000, 2003). In this model, retrograde degeneration of SGNs was seen after central processes of the cochlear nerve were injured using a compression-recording electrode. TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end labeling assay)-positive apoptotic neuronal death and activation of caspase 3 were seen in the spiral ganglion within 5 days after auditory nerve compression, although the pace of SGN death was relatively slow.

8.5 Preservation of the Auditory Nerve

In the past several decades, remarkable progress was made in understanding SGN development and the critical role of neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3), in the maintenance of the auditory nerve during development and adulthood (Goodrich, Chap. 2; Fritzsch, Chap. 3; Rutherford and Moser, Chap. 5; reviews by Fritzsch et al., 2004; Ramekers et al., 2012; Green et al., 2012). Studies have also been conducted to evaluate the protective roles of neurotrophins on the auditory nerve using deafened animal models of SGN degeneration caused by exposure to ototoxic agents (reviews by Gillespie & Shepherd, 2005; Roehm & Hansen, 2005; Budenz et al., 2012). Treatment of deafened animals with exogenous neurotrophin has led to increased SGN survival rate, enlarged SGNs, regrowth of peripheral afferent processes, and auditory function recovery to various degrees. However, many questions remain to be answered before neurotrophin-associated therapeutic approaches can be safely applied to patients with sensorineural hearing loss. Do the neurotrophin-treated nerve fibers change their physiological properties? Do high- and low-SR auditory fibers respond to neurotrophins differently? What are the long-term effects of neurotrophin treatment on the survival of SGNs and related components in the central auditory system? How does the application of neurotrophin with a variety of combinations and concentrations affect the tonotopical gradient of BDNF and NT-3 expression in the surviving SGNs? How do non-neuronal cells such glial cells or endothelial cells respond to the application of neurotrophins?

Angiogenesis as well as ectopic and disorganized sprouting of auditory nerve fibers were seen in deafened cats treated with BDNF and electrical stimulation from a cochlear implant (Leake et al., 2013). Substantial angiogenesis may increase the risk of tumor formation. Disorganization of the auditory nerve outgrowth after neurotrophin treatment also may affect the functional outcome of a cochlear implant.

Proper function of SGNs require a healthy cochlear microenvironment, such as normal levels of EP, intact sensory hair cells and supporting cells, and also structural integrity of IHC/synapses and myelin sheaths provided by cochlear glial cells. Like sensory hair cells, supporting cells can be an important source of neurotrophin crucial to SGN survival (Stankovic et al., 2004; Sugawara et al., 2005). Glial cells, including Schwann and satellite cells in the auditory nerve, are thought to develop from the neural crest. Glial cells in the central nervous system are important in the regulation of tissue homeostasis, shaping synaptic connectivity and controlling adult neurogenesis after injury (Barres, 2008; Rolls et al., 2009). Previous studies indicated a protective role of Schwann cells on the maintenance of the auditory nerve. A reciprocal signaling mechanism has been found in cultured cochlear glial cells, which provide neurotrophic support to SGNs (Hansen et al., 2001). In mice without activation of the receptor tyrosine-protein kinase ErbB2, an essential protein for the development of Schwann cells, abnormal innervation of the organ of Corti is present during inner ear development (Morris et al., 2006). A loss of glial

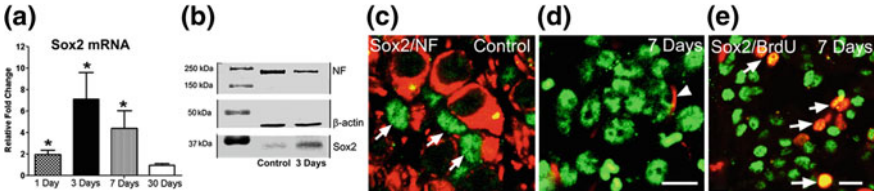

Fig. 8.12 Sox2 upregulation and cell proliferation in the injured adult auditory nerve. **a** Reverse transcriptase-polymerase chain reaction assays show fold changes for Sox2 mRNA expression in the injured auditory nerve. (n = 4–6 per group). **b** Western blot assays show increased Sox2 expression 3 days after ouabain exposure, whereas expression of NF decreased as a result of SGN loss. (**c**, **d**) Dual immunostaining for Sox2 (*green*) and NF (*red*) shows the number of $Sox2^+$ glial cells increase in the auditory nerve 7 days after ouabain exposure. The *arrowhead* indicates a surviving NF^+ SGN process. **e** Dual immunostaining for Sox2 (*green*) and BrdU (*red*) in an injured auditory nerve shows that a majority of $BrdU^+$ cells were Sox2 positive (*arrows*). Scale bars, 7 μm in (**c**, **d**); 12 μm in (**e**) (Figure was modified from Lang et al., 2011)

cells and dysfunction in fibroblast growth factor associated signaling are associated with the degeneration of SGNs and hearing loss (Wang et al., 2009).

Cochlear glial cells most likely are an important resource for the regeneration and self-repair of the adult auditory nerve. Activation of glial cells is associated with neurite growth in a mixed culture of mouse neonatal spiral ganglia (Whitlon et al., 2009). Examination of ouabain-exposed ears revealed a temporal pattern of glial cell phenotypic changes (Lang et al., 2011). Shortly after ouabain exposure, the activation of glial cells was observed in both Rosenthal's canal and osseous spiral lamina. Cell hyperplasia and hypertrophy occurred mostly in the 3 and 7 day postexposure groups. The transcription factor Sox2 is predominantly expressed in proliferating and undifferentiated neural precursors during development and adult neurogenesis in the central nervous system (Ferri et al., 2004; Pevny & Nicolis, 2010). Sox2 is also responsible for the determination of the prosensory domains and the differentiation of SGNs in the developing inner ear (Kiernan et al., 2005; Hume et al., 2007; Dabdoub et al., 2008). Sox2 upregulation and glial cell proliferation occur in the auditory nerve shortly after ouabain exposure (Fig. 8.12; Lang et al., 2011). Dual-staining of Sox2 with a neuronal marker indicates high levels of Sox2 expression in the nuclei of non-neuronal cells. In the injured auditory nerve, $Sox2^+$ cells significantly increased by 3 and 7 days post-treatment. A majority of $BrdU^+$ cells (about 70 %) in ouabain-treated auditory nerve expressed Sox2, and about 6–10 % of $Sox2^+$ cells stained positive with BrdU at 7 days post-treatment, indicating mature glial cells can change their quiescent phenotype and reenter the cell cycle in response to nerve injury. Sox2 is primarly expressed in neural stem cells (NSCs) and plays a critical role in the regulation of neural cell differentiation during development. Although a group of $Sox2^+$ glial-like cells are capable of producing neurons, glial cells, and undifferentiated neural cells in the subgranular zone of the adult hippocampus (Suh et al., 2007), some quiescent glial cells in the adult

auditory nerve also express Sox2. Further study is required to determine whether $Sox2^+$ glial cells are the resource of adult neural stem/progenitor cells (NSPs), like other glial-like NSPs identified in the central nervous system (Suh et al., 2007).

8.6 Summary, Conclusions, and a Few Unanswered Questions

The morphological integrity of the auditory nerve is required for the proper functioning of the mammalian auditory system. Functional and morphological studies conducted over the past several decades have provided insight into a wide spectrum of hearing impairments and related cellular and molecular mechanisms of degeneration of the auditory nerve. Many fundamental questions still remain. For example: how do non-neural cochlear elements contribute to the loss and dysfunction of SGNs? What are the long-term consequences of lateral wall dysfunction resulting in a lowered EP on the survival of SGNs? What is an effective strategy to preserve low-SR fibers or limit the degeneration of the SGNs after noise exposure and with aging? Is the auditory nerve capable of regeneration with the assistance of outside intervention? Do activated cochlear glial cells after auditory nerve injury have neural stem/progenitor properties?

In "Structure of the Mammalian Cochlea" in a previous SHAR volume entitled *The Cochlea*, Dr. Norma B Slepecky (1996, p. 111) wrote, "Anatomical studies on cells of the mammalian inner ear have provided us with many clues as to their different rules in the perception of sound. However, cells do not act in isolation, and hearing depends critically on interactions between cells- some structurally related, others spatially separate but functionally related." -Similarly, SGNs do not act alone; to maintain normal function, SGNs require a healthy cochlear microenvironment comprising many molecular and cellular components. Just as the importance of neural growth factors are to the survival and maintenance of SGN, interaction between SGN and adhesion molecules, extracellular matrix components, cytokines, and the physiochemical nature of the environment including ionic strength are also critical for the survival or death of SGNs with aging or in stress conditions such as noise exposure. Many types of non-neural cells in the inner ear may respond to the survival and death of SGNs. These cells include but are not limited to sensory hair cells, supporting cells, glial cells, cells in the cochlear lateral wall, or inflammatory cells engrafted from bone marrow derived cells. The complex interactions between SGNs and the cochlear microenvironment are important areas for exploration to better understand the mechanisms of SGN degeneration and dysfunction. Recent significant progress in the genetics of sensorineural hearing loss, molecular biology of the developmental auditory nerve, and biophysics of hair cell synaptogenesis, together with the application of systems biology will provide new knowledge and novel methods to answer the fundamental questions of SGN loss, degeneration, and preservation.

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