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Auditory neuropathy — neural and synaptic mechanisms

Tobias Moser¹ and Arnold Starr²

Abstract | Sensorineural hearing impairment is the most common form of hearing loss, and encompasses pathologies of the cochlea and the auditory nerve. Hearing impairment caused by abnormal neural encoding of sound stimuli despite preservation of sensory transduction and amplification by outer hair cells is known as ‘auditory neuropathy’. This term was originally coined for a specific type of hearing impairment affecting speech comprehension beyond changes in audibility: patients with this condition report that they “can hear but cannot understand”. This type of hearing impairment can be caused by damage to the sensory inner hair cells (IHCs), IHC ribbon synapses or spiral ganglion neurons. Human genetic and physiological studies, as well as research on animal models, have recently shown that disrupted IHC ribbon synapse function — resulting from genetic alterations that affect presynaptic glutamate loading of synaptic vesicles, Ca^{2+} influx, or synaptic vesicle exocytosis — leads to hearing impairment termed ‘auditory synaptopathy’. Moreover, animal studies have demonstrated that sound overexposure causes excitotoxic loss of IHC ribbon synapses. This mechanism probably contributes to hearing disorders caused by noise exposure or age-related hearing loss. This Review provides an update on recently elucidated sensory, synaptic and neural mechanisms of hearing impairment, their corresponding clinical findings, and discusses current rehabilitation strategies as well as future therapies.

Auditory neuropathy

A hearing impairment found in individuals with hereditary motor and sensory neuropathy; impairs speech comprehension beyond what would be expected on the basis of pure tone audiograms.

Approximately 360 million people — 5% of the world’s population — have a disabling hearing impairment¹. Hearing impairment can lead to social isolation, depression, and a reduction in professional capabilities. Microsurgery — either alone or combined with innovative implantable hearing aids — successfully tackles conductive hearing loss that arises from disorders of the middle ear. However, sensorineural hearing impairment, which results from disorders of the cochlea and auditory nerve, is more common than conductive hearing loss, and curative pharmacological, gene therapy or stem cell treatments are not yet available for sensorineural hearing impairments. For such therapies to be developed, an improved understanding of the cellular mechanisms of sensorineural hearing impairment is essential. Moreover, advanced genetic diagnostics and clinical audiology are the key to identifying the site of a lesion and to characterizing the specific disease mechanism(s) of sensorineural hearing impairment in an individual patient.

Auditory neuropathy is a nosological term that was coined to describe hearing impairment in individuals with hereditary motor and sensory neuropathy². In auditory neuropathy, the hearing impairment commences downstream from mechano-electrical transduction and cochlear amplification of outer hair cells (OHCs).

Recently, human genetic studies and analysis of animal models of auditory neuropathy have elucidated the wide range of disease mechanisms, which include loss of inner hair cells (IHCs) or IHC synapses, impaired synaptic transmission to spiral ganglion neurons (SGNs), and disrupted propagation of auditory information along the auditory nerve.

In this Review, we provide an update on sensory, synaptic and neural disease mechanisms in auditory neuropathy. We summarize recent insights into the function, dysfunction and excitotoxic loss of IHC ribbon synapses and also discuss disease mechanisms primarily affecting spiral ganglion neurons. We then provide a short guide to clinical diagnosis of auditory neuropathy, with a focus on appropriate physiological and psychophysical tests. Finally, we discuss current strategies for hearing rehabilitation in patients with auditory neuropathy and finish by providing an outlook on future therapeutic options to restore hearing.

Background

Impaired sound encoding in auditory neuropathies

For decades, research into hearing loss focused on dysfunction or loss of sensory hair cells and failure of cochlear ion homeostasis as key mechanisms of sensorineural

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Key points

- Auditory neuropathy impairs speech comprehension severely, beyond the extent that would be expected on the basis of increased threshold of audibility
- Auditory neuropathy encompasses a range of disease mechanisms that typically disrupt the synaptic encoding and/or neural transmission of auditory information in the cochlea and auditory nerve
- Auditory synaptopathy, impaired sound encoding at the synapses between inner hair cells and spiral ganglion neurons, results from genetic defects or insults such as exposure to loud noise
- Advanced physiological and psychophysical testing combined with molecular genetic analysis facilitate diagnostics of auditory synaptopathy and neuropathy
- Although traditional hearing aids often do not provide substantial benefit for patients with auditory synaptopathy or neuropathy, cochlear implants can provide effective hearing rehabilitation depending on the site(s) of disorder

Ribbon synapses

Highly specialized synapses between the inner hair cells and spiral ganglion neurons, with an electron-dense structure — the synaptic ribbon — at the presynaptic active zone that mediates neurotransmitter release.

Cochlear microphone potentials

Outer hair cells generate local cochlear potentials that follow the sound stimulus so precisely that they are called 'microphone potentials'.

Otoacoustic emission

Sound generated from within the inner ear that can be measured with a sensitive microphone in the external ear canal to assess outer hair cell function.

Auditory brainstem responses

Evoked potentials in response to repetitive acoustic stimulation that are recorded from scalp EEG electrodes and typically have five peaks, referred to as waves I–V.

Spiral ganglion compound action potential

The first auditory brainstem response peak, wave I, reflects the spiral ganglion compound action potential; this potential can be recorded with better resolution using electrocochleography.

Auditory synaptopathy

Hearing impairment caused by dysfunction or loss of ribbon synapses in the inner hair cells; has been termed auditory synaptopathy and can show clinical findings similar to those described above for auditory neuropathy.

hearing impairment, with less emphasis on neural conduction by the auditory nerve and encoding of sound at the synaptic level. In the 1990s, Arnold Starr and colleagues described sensorineural hearing impairment in individuals with spared OHC function, as evidenced by preserved cochlear microphone potentials (CMPs) and otoacoustic emissions (OAEs), but abnormal auditory brainstem responses (ABRs) and spiral ganglion compound action potentials that indicate impaired function of the auditory nerve² (FIG. 1). On the basis of pure-tone audiograms, speech recognition was impaired in these patients beyond what would be expected². In addition, the ability of these patients to process temporal cues of acoustic signals was impaired, even though frequency discrimination was preserved³; this temporal encoding deficit impairs sound localization, speech perception and music appreciation. We suggest that the degradation of the temporal neural code is the main substrate of the hearing impairment in auditory synaptopathy and neuropathy (FIG. 2). Moreover, individuals with auditory neuropathy do not typically derive much benefit from hearing aids owing to impaired function of auditory nerve, reflecting their reports that making sounds louder does not improve speech comprehension. In the 2000s, the concept of degraded temporal precision in the neural encoding of sound in spiral ganglions became prominent, and the auditory neuropathy phenotype was more widely reported. The wide range of aetiologies that were suggested by genetic and clinical phenotyping led to the introduction of broader clinical classifications, such as 'auditory dys-synchrony' and 'auditory neuropathy spectrum disorder'. Efforts have been made to reach a consensus on appropriate diagnostics and rehabilitation of hearing^{4–6} in auditory neuropathy.

Prevalence

Unfortunately, considerable uncertainty prevails regarding the prevalence of auditory neuropathy. Estimates of how frequently hearing impairment is caused by auditory neuropathy vary substantially, from $\leq 1\%$ ^{7,8} to almost 10%⁹ of individuals with hearing impairment. Some of this variance is attributed to the inclusion in these studies of populations with different types of hearing impairment.

The reported estimates of the prevalence of genetic auditory neuropathy range from a relatively large prevalence of a single mutation in a specific gene to a single family with one pathogenic mutation in a given gene. For example, the predominant 'Spanish mutation' (Gln829Ter, resulting in truncation) in *OTOF* was found in 8% of the congenital nonsyndromic deaf population with autosomal recessive inheritance of Spanish descent, and is the third-most common cause for prelingual deafness¹⁰. By contrast, a mutation in *SLC17A8* — which encodes vesicular glutamate transporter 3 (VGLUT3) — has been described in only one large family of Czech descent¹¹. Currently, we can only speculate on the extent to which synaptopathic and/or neuropathic disease mechanisms contribute to common forms of hearing impairments, such as noise-induced and age-related hearing loss. However, given the high prevalence of these impairments and the evidence from animal research that synaptopathic mechanisms are involved in noise-induced and age-related hearing loss (discussed below), we should assume auditory neuropathies to have a substantial role.

Sound encoding at the IHC ribbon synapse

Animal research has profoundly advanced our understanding of the disease mechanisms of hearing impairments, particularly those affecting sound encoding at the hair cell ribbon synapses. Ribbon synapses also exist in the retina¹²; indeed, human retinal synaptopathies have been shown to cause stationary night blindness^{13–15}. Moreover, animal models of auditory synaptopathy and neuropathy have shown how disruption of synchronous afferent signalling in the cochlea can account for clinical observations^{16–21}.

IHC ribbon synapses have unique properties

The glutamatergic synapse between IHCs and SGNs is highly specialized to achieve indefatigable afferent transmission at rates of hundreds of Hertz and with sub-millisecond temporal precision. Unlike neurons in the CNS, each bipolar SGN receives input from only one presynaptic active zone of one IHC, which is occupied by an elaborate electron-dense specialization, the synaptic ribbon, that tethers dozens of synaptic vesicles^{12,22} (FIG. 3). Once the mechanical vibration deflects the hair bundle at the apex of the hair cell, mechanotransducer channels open and the ensuing cation influx generates a depolarizing receptor potential, which triggers Ca^{2+} influx through voltage-gated Ca^{2+} channels at the presynaptic active zones of the ribbon synapse, driving synaptic vesicle fusion and glutamate release. Hence, rather than transmitting a binary action potential code, the IHC–SGN ribbon synapse encodes a graded presynaptic signal into a rate code of the SGN. The temporal precision of synaptic transmission is so high that spike-timing in the SGN is locked to the phase of the sound stimulus for frequencies up to approximately 1 kHz^{23,24}.

The active zone of the IHC ribbon synapses differs from active zones of conventional synapses and even from ribbon synapses of the retina in its molecular composition²⁵. Most notably, neither neuronal SNAREs²⁶

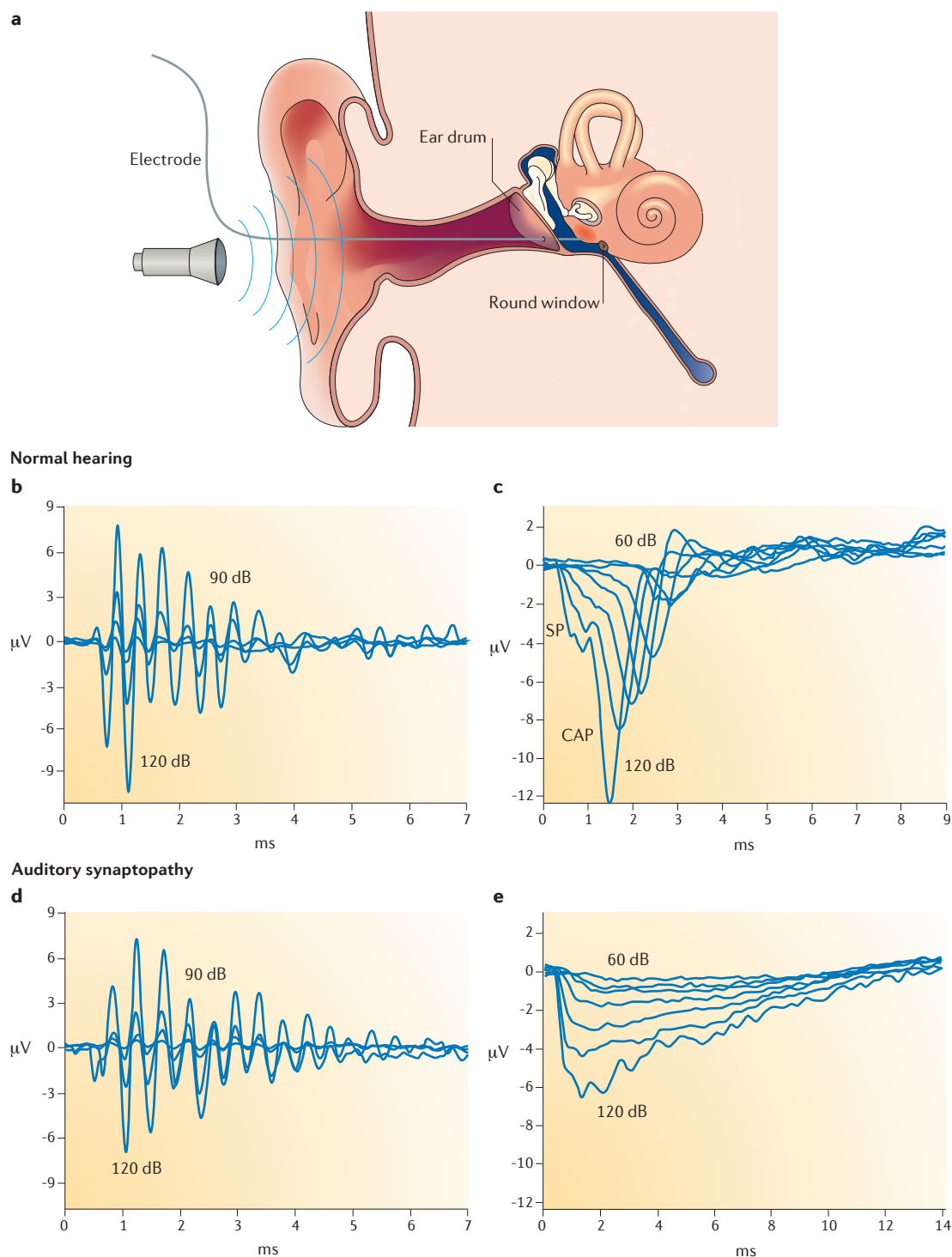


Figure 1 | Clinical neurophysiology for definitive diagnosis of auditory neuropathy. **a** | Transtympanic electrocochleography is straightforward to carry out under local anaesthesia, and provides access to the electrical potentials of the cochlea: microphonic potential (MP, primarily reflecting outer hair cell transduction), summing potential (SP, primarily reflecting the summed inner hair cell receptor potential) and compound action potential (CAP, reflecting the synchronized firing of spiral ganglion neurons) of the spiral ganglion (**b–e**). Example traces obtained in electrocochleography elicited by clicks of increasing volume. **b** | Clicks elicit MPs in an individual with normal hearing at sound pressure levels as low as 90 dB. **c** | Clicks elicit SPs and CAPs in an individual with normal hearing. **d** | In an individual with an auditory synaptopathy caused by *OTOF* mutation, MPs are intact; **e** | SPs are also present, but CAPs are not detectable even for 120 dB clicks, indicating intact outer and inner hair cell sound transduction but lack of synchronous SGN activation, most likely caused by failure of synaptic transmission owing to dysfunctional otoferlin protein. **b–e** adapted with permission from Elsevier Ltd © Santarelli et al. *Hear. Res.* **330**, 200–212 (2015).

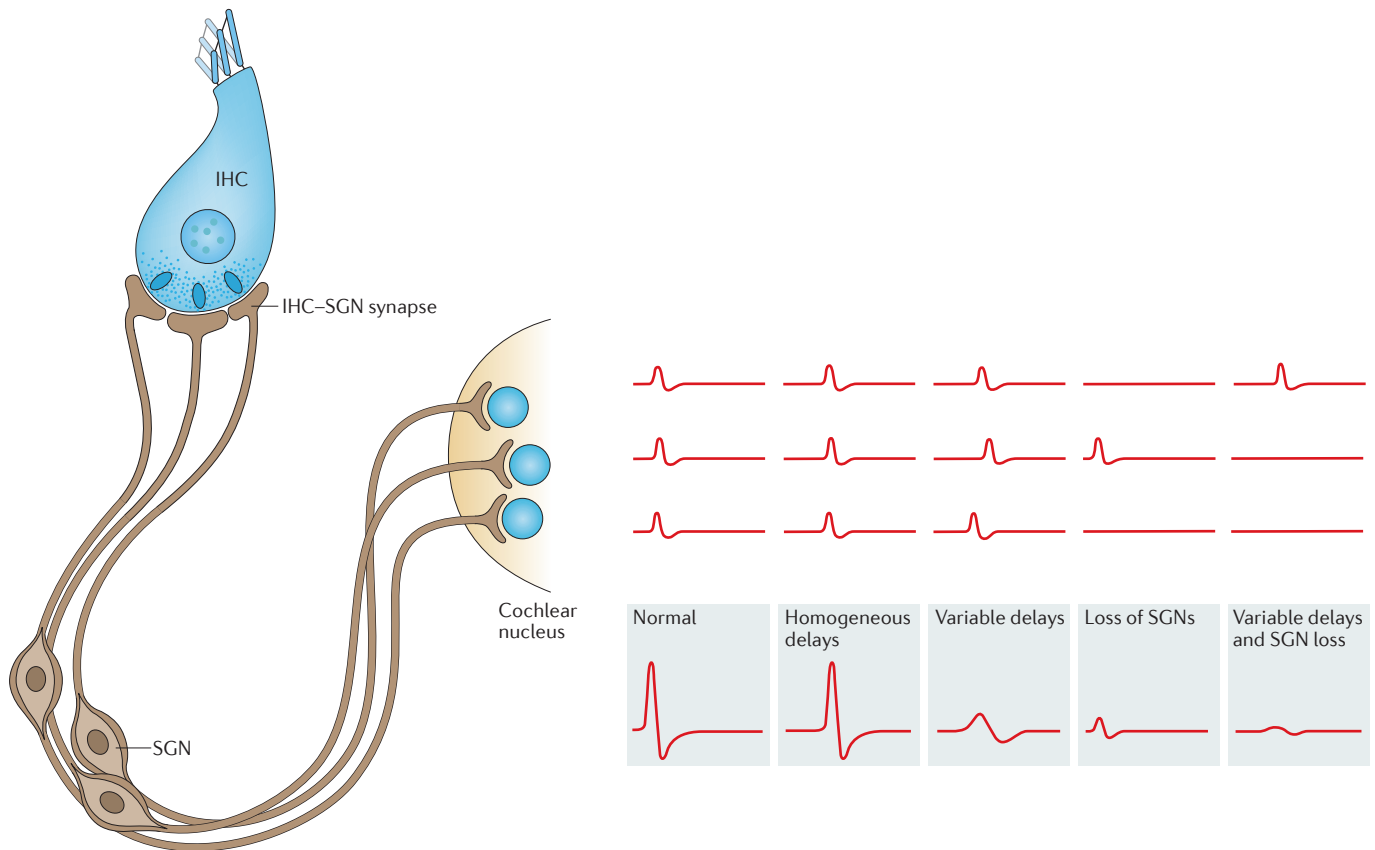


Figure 2 | Disorders affecting inner hair cells (IHCs), their afferent synapses or spiral ganglion neurons (SGNs) degrade the neural representation of sound. **a** | Each IHC forms afferent synapses with approximately 10–30 SGNs, of which each is thought to get input from only one IHC active zone. **b** | Population responses, such as compound action potentials and auditory brain stem responses, arise from summation of the synchronized activity of many SGNs. Increased but homogeneous delays of action potential formation, as seen in conductive hearing loss, increase the latency but do not decrease the maximal amplitudes of the SGN population response. Variable delays of action potentials degrade the amplitude of the population response and also increase latency. Loss of activity, for example as a result of loss of SGNs, decreases the amplitude but does not affect the latency and the combination of variable delays and loss of activity produces the worst SGN population responses, if they persist at all.

(soluble *N*-ethylmaleimide-sensitive-factor attachment receptors), SNARE-regulators such as complexins^{27,28}, nor priming factors of the MUNC13 family²⁹ seem to operate in mature IHCs. Instead, hair cells employ the multi- C_2 -domain protein otoferlin^{19,29,30}, though otoferlin is likely to be only the first identified major player in an orchestra of unconventional synaptic proteins regulating exocytosis at the IHC synapse. Moreover, unlike in conventional glutamatergic presynapses, vesicular glutamate uptake at the IHC synapse is mediated by VGLUT3^{31,32} rather than VGLUT1 or VGLUT2, and Ca^{2+} signalling involves the $Ca_v1.3$ L-type Ca^{2+} channel instead of $Ca_v2.1$ P/Q-type or $Ca_v2.2$ N-type Ca^{2+} channels^{33,34}. Defects in the genes that encode otoferlin, VGLUT3 and the Ca^{2+} channel complex cause human auditory synaptopathy (see below).

Properties of SGNs

SGNs are bipolar neurons that are enwrapped by Schwann cells in the cochlea, and by oligodendrocytes where they enter the brainstem. *In vivo* extracellular recordings of SGN action potentials in response to sound in various

model animals have provided important insight into the role of SGNs in sound encoding^{35,36}. Each SGN is tuned to a specific frequency that best triggers neural activity. This characteristic frequency corresponds to the tonotopic location of the IHC in the organ of Corti that is innervated by the SGN. Interestingly, even SGNs with near-identical characteristic frequencies, which have high likelihood of innervating the same IHC, differ vastly in their rates of spontaneous and sound-evoked firing. The origin of this neural diversity is not well understood, but is likely to involve heterogeneous presynaptic and postsynaptic properties among the hair cell ribbon synapses³⁶.

SGNs exhibit phasic firing, (that is, they fire one or few spikes even in response to long-lasting current injections^{37,38}; this feature is probably attributed to hyperpolarizing K^+ conductances³⁷). SGNs are electrically 'tight' cells and show large excitatory postsynaptic currents³⁹; single presynaptic release events, possibly even the release of a single vesicle⁴⁰, are sufficient to trigger an action potential³⁸. SGN action potentials are most likely generated at the heminode of the peripheral SGN neurite, which is just a few micrometres away from

Organ of Corti

The organ of Corti is the end organ of the sense of hearing that harbours the sensory inner and outer hair cells, as well as afferent and efferent nerve fibres and various types of supporting cells.

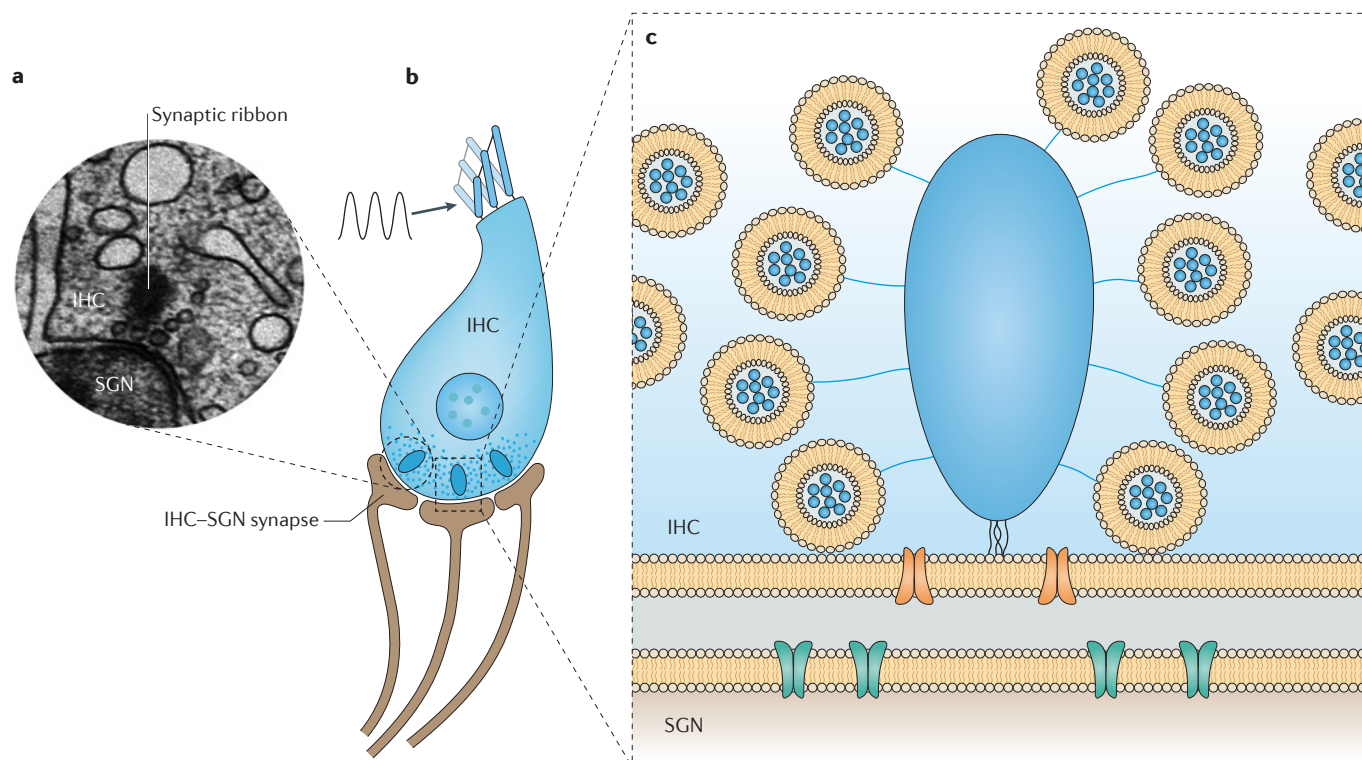


Figure 3 | The afferent ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs).

The ribbon synapses are highly specialized for indefatigable encoding of sound information with submillisecond temporal transmission. **a** | Transmission electron micrograph of a ribbon synapse formed by the presynaptic IHC and the postsynaptic SGN. Note the electron-dense synaptic ribbon tethering synaptic vesicle (small black-rimmed spheres). Image courtesy of Dr C. Wichmann, Molecular Architecture of Synapses group, Institute for Auditory Neuroscience, University Medical Center Göttingen, Göttingen, Germany. **b** | Schematic of an IHC forming several ribbon synapses, each connected to a SGN with distinct spontaneous rate, sound threshold and dynamic range. **c** | Schematic of the IHC ribbon synapse. The synaptic ribbon is anchored to the presynaptic membrane by large scaffolding proteins. The molecular composition of the IHC ribbon synapse vastly deviates from that of glutamatergic synapses of the CNS. **b, c** are courtesy of Dr R. Nouvian, Institute for Neurosciences of Montpellier, France.

the IHC synapse³⁸. Similar to the downstream complete nodes of Ranvier, the heminode that precedes the myelin sheath shows a high density of voltage-gated Na^+ (Na_v) channels^{21,41} that, together with passive electrical properties of the distal neurite, enable the invading excitatory postsynaptic potentials to readily trigger spike generation in the SGN³⁸. This mechanism underlies the high temporal precision of sound encoding that is required for detecting temporal cues of acoustic stimuli. Impairments of IHC transmitter release, impairment of spike generation in or spike propagation along SGNs, and altered synaptic transmission from SGNs to neurons in the cochlear nucleus all degrade the signalling of auditory information to the brain. The resulting loss of temporal precision (or synchrony) and inaccurate neural representation of the auditory signal (caused by the loss of synapses and/or neurons) are thought to represent key disease mechanisms in auditory synaptopathy and neuropathy (see below).

Disease mechanisms

Here, we review known mechanisms of genetic and acquired auditory synaptopathy and neuropathy, including disruption of IHCs, the SGNs and the ribbon synapse.

We combine information on human genetics and focus on disorders for which analysis of auditory function in animal models has provided insights into the disease mechanism. We will cover genetic disorders related to otoferlin, VGluT3 , the Ca^{2+} channel complex and *OPA1*. Finally, we will review recent findings on how hypoxia, hyperbilirubinaemia, thiamin deficiency, loud noise and ageing affect IHCs, synapses and SGNs, again building on data from animal models and the first observations in humans.

Genetic auditory synaptopathies

Otoferlin genetics, physiology and pathophysiology. *OTOF*, the gene coding for otoferlin, was among the first genes identified as being affected in autosomal recessive sensorineural deafness ('deafness, nonsyndromic autosomal recessive 9'; DFNB9)⁴². DFNB9 is a severe to profound prelingual hearing impairment in which none of the symptoms arise from dysfunction of other senses or the brain. It has the audiological signature of auditory neuropathy, and was suspected to reflect a defect of pre-synaptic function in inner hair cells⁴² soon after its characterization. During the past decade, rare cases of less severe otoferlin-related auditory synaptopathies have

been reported^{43–47}. In some patients with these disorders, an elevation in body temperature (even by only 1°C) as a result of exercise or fever^{43–47} can exacerbate the condition into complete deafness accompanied by tinnitus. At normal body temperature, audibility can be close to normal in these patients, though their speech recognition is impaired, particularly in background noise.

Otoferlin is expressed in sensory hair cells and belongs to the ferlin family, which also includes the muscle proteins dysferlin and myoferlin. Ferlins have been implicated in membrane fusion events^{25,48} and are of great interest to neurologists for reasons beyond their role in otoferlin-related hearing disorders. Dysferlin, for example, is defective in limb-girdle muscle dystrophy type 2B⁴⁹, which has been linked with impaired membrane resealing in skeletal muscle cells — a process that requires dysferlin-dependent fusion of membranous organelles with the plasma membrane⁵⁰.

Like the other ferlins, otoferlin contains a ferlin-specific motif of unknown function, a coiled-coil domain that might form a helical bundle with other coiled-coil domains, and six or seven C₂ domains⁵¹, which generally bind to Ca²⁺ and phospholipids and/or interact with other proteins. Several C₂ domains of otoferlin have been implicated in Ca²⁺ and phospholipid binding, though controversy still remains regarding which of the C₂ domains contribute to binding, and how^{19,30,52–54}. The sequence similarity between ferlin C₂ domains and other C₂ domain proteins, such as protein kinase C or synaptotagmin, is low, making it difficult to infer the structural determinants of otoferlin binding to Ca²⁺ and phospholipid from those of better-characterized proteins²⁵. We are only beginning to obtain insight into the protein structure of ferlins^{55,56}.

To date, approximately 90 pathogenic mutations of *OTOF* have been reported; the majority of these mutations are assumed to be nonsense or truncation mutations that cause inactivation of otoferlin⁵⁷, resulting in DFNB9-type hearing impairment. Otoferlin knock-out mice are likely to be an appropriate animal model for DFNB9 (REFS 19,30). Indeed, Ca²⁺-triggered exocytosis is nearly abolished in IHCs of these mice^{19,30}; synaptic vesicles were found close to the membrane at the active zone, suggesting that an absence of vesicles was not limiting signal transduction, but a late step of exocytosis was disrupted³⁰. This finding, together with biochemical evidence for a role of otoferlin in Ca²⁺ and phospholipid binding (see above) support the hypothesis that otoferlin serves as a Ca²⁺ sensor in exocytosis in hair cells, as do synaptotagmins 1 and 2 in conventional synapses (FIG. 4). However, a rescue experiment in which synaptotagmin 1 was expressed in otoferlin-deficient IHCs failed to restore IHC exocytosis or hearing⁵⁸. This might reflect the absence of appropriate interaction partners of synaptotagmin (such as neuronal SNAREs) that are required for its function. Interestingly, otoferlin knock-out mice showed a more subtle vestibular dysfunction, both at the hair cell and systems level⁵⁹. This finding is in line with clinical reports of normal vestibular function in patients with DFNB9 (REF. 60).

Missense mutations cluster in the regions of DNA that encode the C₂ domains of otoferlin and are likely to impair their function; such mutations can also reduce the otoferlin levels in hair cells¹⁹. To date, six *OTOF* mutations have been associated with temperature-sensitive deafness^{43–47}. The ‘*pachanga*’ missense otoferlin mouse mutant, generated in a forward genetic screen⁶¹, provided important additional insight into the function of otoferlin. In the IHCs of these mutant mice, exocytosis was normal in response to short stimuli, but much reduced as soon as vesicle replenishment was required at the release sites of the active zone¹⁹. This defect in vesicle replenishment was shown to nearly abolish sound encoding at the hair cell synapse *in vivo*. Only the highest sound pressure levels applied at a low stimulus rate triggered spikes in SGNs at the single neuron level, but population responses of the spiral ganglion (as assessed by ABRs and electrocochleography, which is a powerful diagnostic tool to help identify the site of lesion) remained undetectable. Consistent with a failure in efficient vesicle replenishment for indefatigable transmission, psychophysical and physiological tests revealed progressive failure of afferent signalling (auditory fatigue) in humans with a missense mutation in *OTOF*⁶².

Although the precise mechanism by which mutation impairs the function of otoferlin at hair cell synapses remains to be elucidated, three interesting hypotheses for the role of otoferlin in vesicle replenishment have been suggested (FIG. 4). Otoferlin could prime synaptic vesicles for fusion¹⁹, potentially via promoting the formation of short tethers between synaptic vesicles and the membrane of the active zone, as suggested by electron tomography²⁹, and/or it could facilitate the clearance of previously exocytosed material from the release site via interaction with endocytic proteins^{18,63}. Missense mutations might affect these processes by lowering the otoferlin levels or by impairing its interactions with other proteins. Indeed, in *pachanga* mice, the levels of otoferlin were drastically reduced in the IHCs, particularly at the plasma membrane¹⁹. This finding could be representative of some, if not all, human otoferlin missense mutations. The mechanisms through which elevated temperature triggers deafness and tinnitus are yet to be explored, but an additional reduction of otoferlin levels might be involved.

Further research on otoferlin is central to understanding the molecular physiology and pathophysiology of synaptic sound encoding. Site-directed mutagenesis will require more structural information and biochemistry, as well as efficient mutagenesis. Identification of additional interaction partners of otoferlin will be instrumental in dissecting the molecular machinery at the hair cell ribbon synapse.

Vesicular glutamate transporter 3. A mutation in locus DFNA25 on 12q21–24 that causes nonsyndromic autosomal dominant hearing impairment was first described in 2001 (REF. 64), and the genetic defect was further characterized¹¹. The affected gene in DFNA25 is *Slc17a8* (REF. 32), resulting in dysfunctional VGLUT3.

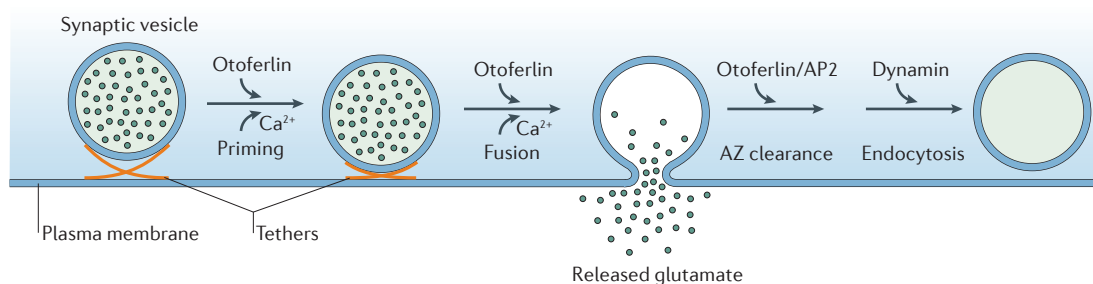


Figure 4 | Otoferlin has an essential role at the inner hair cell (IHC) ribbon synapse. Otoferlin is involved in vesicle fusion with the plasma membrane, and in vesicle replenishment. The role of otoferlin in vesicle replenishment might involve tethering as well as interaction with endocytic proteins, such as adaptor protein 2 (AP2) that enables rapid clearance of exocytosed material from the vesicular release site. AZ, active zone. Adapted with permission from Elsevier Ltd © Pangršić et al. *Trends Neurosci.* **35**, 671–680 (2012).

A series of studies demonstrated strong expression of and a strict requirement for VGLUT3 in afferent synaptic transmission in hair cells of mouse^{31,32} and zebrafish⁶⁵ (FIG. 5). IHCs lacking VGLUT3 exhibited normal Ca^{2+} influx and synaptic vesicle cycling³². However, there was no excitatory afferent synaptic transmission to SGNs³¹, and sound-evoked activation of the auditory pathway was absent^{31,32} despite normal³² or only mildly altered³¹ presynaptic morphology.

The precise disease mechanism through which the mutated VGLUT3 protein causes hearing impairment remains to be elucidated. A haploinsufficiency has been postulated, based on reports of individuals with a major deletion in the DFNA25 locus⁶⁶ that probably results in a non-functional *SLC17A8* allele. Alternatively, a gain-of-function mutation could lead to enhanced transport that might make IHC synapses prone to excitotoxic synapse loss (see section on acquired auditory synaptopathies) owing to an increase of the vesicular glutamate load. Moreover, the question of whether hearing impairment associated with *SLC17A8* mutations is non-syndromic requires reconsideration, given that hearing impairment along with mental and motor retardation was observed in an individual with a probable *SLC17A8* mutation⁶⁶, and mice globally lacking *Slc17a8* have seizures, a sign of a generalized CNS disturbance³¹.

Ca^{2+} channel complex. A syndromic deafness in humans has been associated with a loss-of-function mutation in the *CACNA1D* gene, which codes for the pore-forming $\alpha 1$ subunit of the $Ca_v1.3$ Ca^{2+} channel⁶⁷ that is expressed in hair cells, cardiomyocytes, neurons and neuroendocrine cells. $Ca_v1.3$ Ca^{2+} channels are involved in diastolic depolarization in cardiomyocytes of the sinoatrial node, and mediate more than 90% of the voltage-gated Ca^{2+} influx in IHCs^{33,34}. Accordingly, individuals with the mutation exhibit sinoatrial node dysfunction and deafness (SANND) syndrome⁶⁷. On the basis of animal studies, $Ca_v1.3$ channels have been implicated in neurotoxicity of dopaminergic substantia nigra neurons⁶⁸ and in fear memory⁶⁹. Individuals with SANND did not report neuropsychiatric symptoms, but a detailed assessment is yet to be performed to explore possible CNS effects of $Ca_v1.3$ abolition⁶⁷ and potential endocrine imbalances caused by $Ca_v1.3$ disruption.

The first mutation to be associated with SANND (insertion of a glycine near the pore-encoding region) blocked conduction of the $Ca_v1.3$ channel, as demonstrated in a heterologous expression system⁶⁷. Loss of $Ca_v1.3$ -mediated Ca^{2+} influx in $Ca_v1.3$ knock-out mice disrupts stimulus–secretion coupling at the hair cell active zone³⁴, resulting in deafness³³. To date, deafness associated with mutations in the genes for the auxiliary subunits of the hair cell $Ca_v1.3$ Ca^{2+} channel has not been described. A mouse mutagenesis study revealed that the $\beta 2$ -subunit ($Ca_v\beta 2$) of this channel is required for hair cell Ca^{2+} influx and hearing⁷⁰. The $\alpha 2\delta$ subunit(s) that are expressed by hair cells remain to be determined.

Interestingly, various genetic retinal disorders result from mutations in genes that code for the subunits of the presynaptic $Ca_v1.4$ Ca^{2+} channel of photoreceptors. Such nonsyndromic retinal synaptopathies include congenital stationary night blindness type 2 (REFS 13,14) and autosomal recessive cone dystrophy⁷¹. Additional links between hearing impairment and vision disorders resulting from disruption of the Ca^{2+} channel complex at ribbon synapses are provided by interaction partners that regulate the abundance and function of the channel. In humans, for example, EF-hand Ca^{2+} -binding proteins (CABPs) are affected in congenital stationary night blindness type 2 (attributed to altered CABP4)¹⁵ and autosomal recessive hearing impairment DFNB93 (attributed to altered CABP2)⁷². CABPs are thought to interact and modulate the pore-forming $\alpha 1$ subunit of the channel, shifting the voltage-dependence of their activation to negative potentials⁷³ and antagonizing Ca^{2+} -dependent inactivation⁷². In the retina, lack of CABP4 could impair $Ca_v1.4$ Ca^{2+} channel activation by shifting its operating range outside the physiological receptor potentials of photoreceptors⁷³. In DFNB93, a splice site mutation is assumed to induce skipping of exon 6 of *CABP2*, resulting in a truncated protein that lacks the C-terminal EF-hand Ca^{2+} -binding sites 3 and 4 (REF. 72). This truncated protein shows altered Ca^{2+} binding and antagonizes Ca^{2+} -dependent inactivation of $Ca_v1.3$ less efficiently. Experiments on *Cabp2* mutant mice are needed to fully comprehend the effects of CABP2 disruption on the hair cell Ca^{2+} influx and synaptic sound encoding.

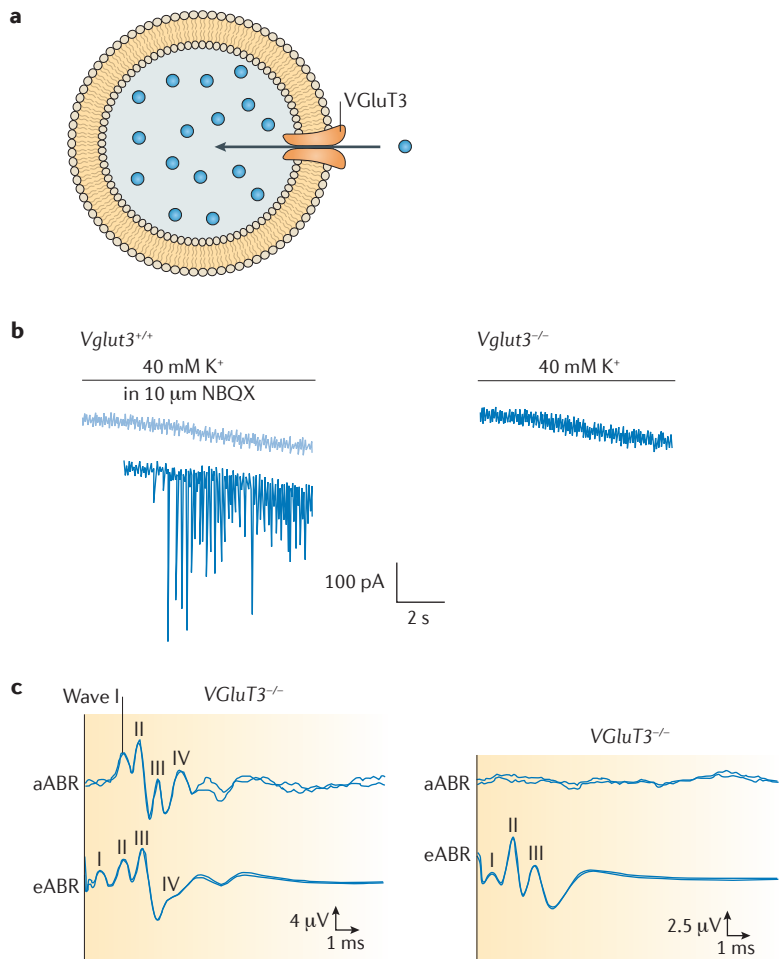


Figure 5 | Hair cells use the vesicular glutamate transporter 3 to load synaptic vesicles with glutamate. **a** | Vesicular glutamate transporter 3 (VGLUT3, red) loads inner hair cell (IHC) synaptic vesicles with glutamate (blue spheres). Image courtesy of Dr R. Nouvian, Institute for Neurosciences of Montpellier, France. **b** | Patch-clamp recordings from postsynaptic spiral ganglion neuron (SGN) terminals in wild-type mice (left) show a barrage of excitatory postsynaptic currents (EPSCs, black traces) upon depolarization of the presynaptic IHC induced by elevated K^+ concentration; these EPSCs are blocked by the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor inhibitor NBQX (grey trace). No EPSCs are observed in *Vglut3*-knockout mice (right). Adapted with permission from Elsevier Ltd © Seal, R. P. et al. *Neuron* **57**, 263–275 (2007). **c** | Auditory brain stem responses (ABRs), triggered acoustically (aABRs) or electrically (eABRs), are seen in wild-type mice (left); in *Vglut3*-knockout mice (right), only eABRs persist. Adapted with permission from the American Society of Human Genetics © Ruel, J. et al. *Am. J. Hum. Genet.* **83**, 278–292 (2008).

Genetic auditory neuropathies

Mutations in optic atrophy 1 (OPA1). Neuropathic hearing impairments are common findings among individuals with syndromic dominant optic atrophy caused by mutations in the gene *optic atrophy 1 (OPA1)*^{74,75}. Dominant optic atrophy is one of the most frequent genetic optic atrophies, and *OPA1* mutations are causal in the majority of the cases⁷⁶. The *OPA1* protein is a mitochondria-targeted dynamin-related GTPase^{77,78} that is critical for mitochondrial shaping⁷⁹. The majority of the ~200 known human *OPA1* mutations (www.mitodyn.org; the MITOchondrial DYNamics variation portal) cause haploinsufficiency via truncation; these mutations are thought to cause nonsyndromic autosomal dominant

optic atrophy (DOA)⁸⁰. Missense mutations, probably acting via dominant negative action of the mutant protein⁷⁵, are considered to cause syndromic autosomal dominant optic atrophy (DOA+), which often (in 60% of patients) also involves hearing impairment, sensorimotor neuropathy, myopathy and ataxia⁷⁵.

DOA+ related hearing impairment often results from the Arg445His missense mutation in *OPA1*, and typically follows the onset of visual impairment with slow progression⁷⁵. Detailed audiological assessment with transtympanic electrocochleography (FIG. 1) indicates normal function of IHCs and OHCs, but a failure of SGN activation in the cochlea, indicative of auditory neuropathy⁷⁴. On the basis of the specific pattern of remaining neural responses, DOA+ related hearing impairment was postulated to result from degeneration of the peripheral neurite of the SGNs. Indeed, most of the individuals with DOA+ who received a cochlear implant had very good rehabilitation outcomes⁷⁴. To date, no hearing deficits have been reported in *Opa1* mutant mice, which carry a splice site mutation that probably causes DOA via haploinsufficiency⁸¹. More-detailed investigation of the cellular mechanism(s) of *OPA1*-related hearing impairment requires the generation of appropriate mouse models.

Hereditary sensorimotor neuropathies affecting the auditory system. The term auditory neuropathy was originally coined to describe sensorineural hearing impairment in individuals affected by hereditary sensorimotor neuropathies (HSMN)⁸². Rather than providing a comprehensive review of HSMN and hearing impairment, we highlight a few examples for which some mechanistic information has been obtained from clinical and animal studies.

Several mutations in the *MPZ* gene, coding for myelin protein zero in Schwann cells, have been shown to cause auditory neuropathy^{83,84}. In one patient, a histological analysis of the SGNs in the temporal bone and other sensory nerves demonstrated a consistent demyelination and neuronal loss across the nerves⁸⁴. Mutations in the *PMP22* gene, including duplication (causing Charcot-Marie-Tooth polyneuropathy type 1a), have been found to cause auditory neuropathy and sensory hearing impairment^{85–87}.

DFNB59-related hearing impairments. Mutations in *DFNB59* have been suggested to result in auditory neuropathy⁸⁸. The protein coded by *DFNB59*, pejvakin (the name derived from the Persian word for ‘echo’), is a member of the gasdermin family and was found in hair cells and neurons of the afferent auditory pathway^{61,88}. The interpretation of pejvakin-associated hearing impairment as neuropathic was based on pathological synchronized spontaneous otoacoustic emissions (ABRs and OAEs) in both human patients and in a *Dfnb59* knock-in mouse that reproduces the human Arg183Trp substitution⁸⁸. However, a pejvakin mouse mutant, generated in a random mutagenesis screen, lacked otoacoustic emissions, which together with evidence of pejvakin expression in OHCs and IHCs was interpreted as support for a role of pejvakin in OHCs⁶¹. Hearing impairment without

otoacoustic emissions was also observed in human patients with *DFNB59* mutations^{89–92}, indicating that *DFNB59* mutations primarily or secondarily also affect cochlear amplification. A recent study has identified a role of pevjakin in the proliferation of peroxisomes in hair cells and SGNs in response to loud sound⁹³. Peroxisomes proliferate as part of the antioxidant response of these cells during acoustic overstimulation, but deletion of pevjakin in mice abolished this response and was associated with greater oxidative damage in the organ of Corti.

DIAPH3-associated auditory neuropathy. Autosomal dominant auditory neuropathy 1 (AUNA1)^{94,95} is thought to result from overexpression of diaphanous homologue 3 (*DIAPH3*)^{96,97}. Although more work is needed to elucidate the function of *DIAPH3* in hair cells and hearing, mouse lines overexpressing *Diaph3* in hair cells provide some interesting clues about its function: in these mutants, the hair bundle morphology of IHCs, but not of OHCs, was disrupted⁹⁷, underlining the fact that the audiological signature of auditory neuropathy can reveal defects in auditory signalling at a stage as early as IHC transduction. Individuals with AUNA1 have not yet been assessed with electrocochleography. If the hypothesis of defective IHC transduction were correct, electrocochleography would reveal impairment or absence of the summing potential, which reflects the summed receptor potential of IHCs. Finally, syndromic neuropathies, such as Friedreich ataxia, also show the clinical features of an auditory neuropathy⁹⁸.

Acquired auditory synaptopathies

Hearing impairment in preterm infants. Neuropathic hearing impairment is common in preterm infants⁹⁹. Preterm infants are much more likely to have sensorineural hearing loss (1 in 50) than are normal-term neonates (1 in 1000)¹⁰⁰. Risk factors for hearing loss include the need for intensive neonatal care¹⁰⁰, and hyperbilirubinaemia^{99,101–103}. Nearly all individuals who did not survive in neonatal intensive care units failed ABR screening, and postmortem analysis revealed bilateral hair cell loss in several infants (selectively in IHCs in three of 15), whereas the number of SGNs was not reduced¹⁰⁴.

The auditory system is particularly sensitive to hyperbilirubinaemia¹⁰³. The precise disease mechanisms of bilirubin neurotoxicity are not yet well understood. The jaundiced Gunn rat that carries a mutation in the gene encoding UDP-glucuronosyltransferase 1 is an animal model for human hyperbilirubinaemia¹⁰⁵. Moreover, application of bilirubin has been used to study acute effects of bilirubinaemia¹⁰⁶. Hypotheses on bilirubin toxicity in the auditory system include damage to presynaptic terminals via excessive nitric oxide¹⁰⁶ and changes in neuronal Ca²⁺ homeostasis¹⁰⁷.

IHC loss underlies thiamine-deficiency-related auditory neuropathy. Thiamine deficiency has been demonstrated to cause auditory neuropathy in children¹⁰⁸. Thiamine-deficiency-related hearing impairment was characterized in detail in Israeli infants who were fed a thiamine-deficient formula. Most of these infants showed auditory

neuropathy, which resolved with supplementary thiamine in five of eight children; two of these infants lacked otoacoustic emissions, suggesting OHC dysfunction¹⁰⁸.

The importance of thiamine for cochlear function was convincingly demonstrated in studies of mice that lacked high-affinity thiamine transporter, which is encoded by *Slc19a2* (REFS 109,110). In one of these studies, a massive and rapid loss of IHCs was observed upon dietary thiamine restriction¹¹⁰. Although IHC loss associated with thiamine deficiency can explain an auditory synaptopathy phenotype, it seems incompatible with the transient nature of the hearing loss observed in infants with thiamine deficiency¹⁰⁸. Moreover, the thiamine-related hearing impairment was present in the absence of major IHC loss¹⁰⁹. Therefore, we consider it likely that thiamine deficiency impairs IHC function before causing loss of IHCs. In conclusion, thiamine deficiency can cause auditory neuropathy, but the disease mechanisms in hearing impairment associated with thiamine deficiency await further analysis in appropriate animal models¹¹⁰.

Noise-induced and age-related hearing loss. In noise-induced, drug-induced and age-related hearing loss, several mechanisms lead to dysfunction or loss of both IHCs and OHCs¹¹¹. These types of hearing impairments typically affect OHCs (as implicated by the lack of otoacoustic emissions). Damage and loss of afferent IHC synapses has been implicated as a contributor to noise-induced and age-related hearing loss, which are the two most common forms of hearing impairment^{112–117}. Indeed, certain noise exposure protocols can cause a temporary threshold shift with full recovery of otoacoustic emissions (OAEs) and ABR thresholds, but a persistent substantial reduction of SGN compound action potential (CAP) amplitudes. Glutamate excitotoxicity that eventually leads to damage or loss of the postsynaptic terminal of SGNs is likely to underlie the synaptic loss in both disorders¹¹⁸ (FIG. 6). Indeed, agonists of ionotropic glutamate receptors damage the IHC afferent synapse¹¹³, and mice lacking the glial glutamate–aspartate transporter GLAST show enhanced susceptibility to noise-induced hearing loss¹¹⁹.

In the past decade, the analysis of excitotoxic synaptic damage has been greatly facilitated by quantitative light microscopy¹²⁰. Counting immunolabeled IHC ribbon synapses in whole-mounted mouse organs of Corti¹¹⁶, Kujawa and Liberman demonstrated a massive loss of ribbon-occupied IHC synapses in the high-frequency cochlea of rodents, even as a result of sound exposures that do not cause a permanent increase in hearing thresholds¹¹⁶ (FIG. 6). The loss of presynaptic ribbons was paralleled by a reduction in the first wave of ABRs that represents the SGN compound action potential¹¹⁶. The fact that the threshold recovered to normal levels was attributed to the recovery of cochlear function upstream of IHC synapses, and the unexpected resistance of synapses formed with SGNs that have low thresholds and high spontaneous rates to the noise exposure, as shown by *in vivo* recordings from single SGNs in guinea pigs¹²¹. Interestingly, a selective loss of low spontaneous rate SGNs was also observed upon ouabain poisoning of the spiral ganglion in gerbils¹²².

Compound action potential
Reflects the synchronized firing of spiral ganglion neurons; assessed by intrameatal or transtympanic electrocochleography.

Glutamate excitotoxicity
Excessive presynaptic glutamate release leading to massive depolarization and subsequent synapse loss.

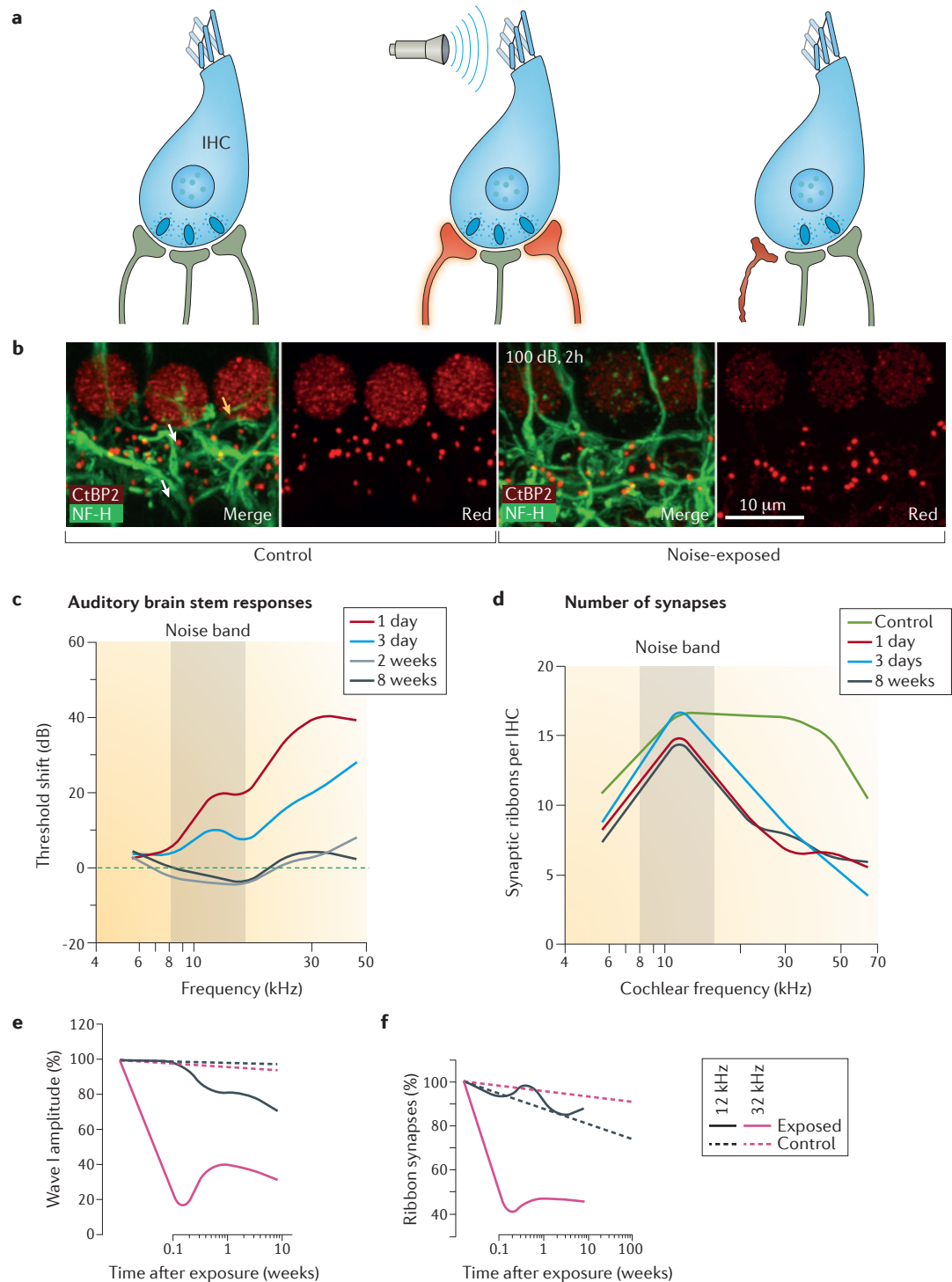


Figure 6 | Irreversible loss of inner hair cell (IHC) ribbon synapses during exposure to loud noise in mice.

a | Rodent studies have demonstrated that noise-induced synaptic damage, likely resulting from excessive release of glutamate from the IHC presynaptic active zone, leads to irreversible loss of IHC–SGN synapses. **b** | Confocal micrographs showing the region of organ of Corti show reduced numbers of IHC synaptic ribbons (red spots) in 16-week old mice 2 weeks after 2-hour noise exposure (8–16 Hz, 100 dB; right) compared with mice not exposed to noise (controls). Sections are stained for neurofilament (green, staining afferent SGN nerve fibres) and RIBEYE/CtBP2 (red, stains synaptic ribbons that are seen as small, intensely fluorescent spots) and IHC nuclei (red, large round weakly fluorescent structures). Image courtesy of S. Kujawa, Massachusetts Eye and Ear, Harvard Medical School, Boston, USA. Mouse studies have shown that noise exposures that lead to only temporary increases in hearing threshold (**c**) can cause an irreversible loss of synaptic ribbons and postsynaptic terminals in the basal cochlea (**d, e, f**), which mediate high-frequency hearing, which is also reflected in a reduced amplitude of the compound action potential¹¹⁶. CtBP2, C-terminal binding protein.

After noise exposure, the damaged peripheral neurites do not form new IHC synapses, as described after a pharmacological excitotoxic insult in the guinea pig cochlea¹¹⁴, but the reasons for injury-specific lack of recovery are not clear. Lack of synapse regeneration after noise might reflect impaired neurotrophic signalling in the organ of Corti¹²³; indeed, synaptic loss (deafferentiation) could be mitigated by virally mediated expression of neurotrophic factors in the mouse cochlea¹²³. Synaptic loss also takes place in the cochlea as a result of ageing in mice^{115,117,118} and is accelerated if animals were previously subjected to 'non-damaging' noise exposure¹¹⁸.

Currently, the role of excitotoxic damage to synapses in hearing impairment has only been studied in animal models; hence, we do not yet know with certainty how relevant excitotoxic synaptic loss is to noise-induced and age-related hearing impairment in humans. If it is relevant, the selective deafferentiation of the SGNs with low spontaneous firing rates and high sound thresholds, which are most susceptible to excitotoxicity, would be likely to cause problems with speech comprehension under noisy conditions, whereas normal audibility would be maintained by the most resistant SGNs with high spontaneous rates and low thresholds. Compound action potentials would be expected to remain, albeit reduced in amplitude. The resulting disorder would be assumed to represent a mild form of auditory synaptopathy with normal pure tone thresholds and speech comprehension in quiet environments. Clinical studies, including electrocochleography and assessment of speech comprehension in noisy environments, are necessary to clarify the relevance and expression of excitotoxic synaptic loss in humans. However, even before such confirmation, the animal studies highlight the importance of limiting occupational and other noise exposure in humans and indicate that pure tone audiometry that solely tests audibility, as often performed in otolaryngology, is insufficient to reveal such 'hidden hearing loss' (REF. 124).

Diagnostics and clinical findings

Clinical features and testing strategies

Clinical features of auditory neuropathy differ from features of disorders that primarily affect cochlear amplification. Psychophysically, they include poor speech recognition, which is affected beyond the extent expected on the basis of the auditory sensitivity reported by pure tone audiometry. If the patients are able to recognize some speech, recognition is typically severely affected by background noise. Pure tone thresholds vary from near normal (in hearing impairments associated with Ca^{2+} -binding protein 2 or certain otoferlin missense mutations) to strongly elevated (for example, in prelingual otoferlin-related deafness DFNB9). Moreover, pathological loudness adaptation (assessed with Carhart's tone decay test) and poor temporal processing (assessed with the gap detection test or by discrimination of low frequencies) indicate dysfunction of synaptic or neural auditory processing in the cochlea (TABLE 1).

Different strategies of audiological testing are recommended according to the feasibility of psychoacoustic testing at the specific age of a patient (TABLE 1). Physiological

testing (primarily ABRs, otoacoustic emissions, such as DPOAEs and transitory evoked OAEs, and electrocochleography (transtympanic recordings are preferable because of a better signal-to-noise ratio than with intrameatal recordings)) is most relevant, but results need to be confirmed by psychophysical testing in all age groups. Accurate diagnosis is important, because the decision to carry out cochlear implantation needs sufficient certainty of diagnosis.

The most important physiological findings in auditory neuropathy^{2,125,126} are impaired or absent compound action potentials and ABRs despite evidence of OHC function, which can be observed by using cochlear microphonics (intrameatal or transtympanic electrocochleography) and/or measuring otoacoustic emissions. In addition, the middle ear (stapedial) reflex and the contralateral suppression of otoacoustic emissions are typically lacking. Electrocochleography and tests of neural adaptation can help site-specific diagnostics beyond discriminating auditory neuropathy from other forms of sensorineural hearing impairment⁸⁷. For example, the absence of the summating potential in electrocochleography indicates the lack of IHC mechano-electrical transduction or absence of IHCs, as is seen in thiamine deficiency or perinatal hypoxia.

Distinguishing neuropathy and synaptopathy

Differentiating between synaptic dysfunction and neuropathy remains challenging, even with advanced analysis. For example, electrocochleography results in similar findings for patients with *OPA1*-associated auditory neuropathy and *OTOF*-associated auditory synaptopathy^{74,127}. Moreover, in animal models of auditory synaptopathy and neuropathy, the delayed and broadened compound action potentials can arise from either presynaptic^{16,17,128} or postsynaptic²¹ defects. Assessing the extent of adaptation to pure tones, on the other hand, might facilitate differentiation between synaptopathy and neuropathy. In otoferlin-associated auditory synaptopathy, adaptation for both low and high frequency tones has been reported to be strongly enhanced⁶². By contrast, patients with auditory neuropathy adapted in a normal manner to low frequencies but showed abnormal adaptation to high-frequency sounds⁶². Overall, individuals with auditory synaptopathy showed faster and more extensive adaptation than those with neuropathy⁶².

Clearly, manifestation of neuropathy or other neurological disorders beyond the auditory nerve is suggestive of primary neural dysfunction. In order to assess the scope of the disorder, it is of utmost importance to combine the detailed audiological analysis with vestibular testing, neuroradiological analysis and clinical neurological and ophthalmological investigation. Genetic analysis is of great help, given the heterogeneous aetiology and, in most cases, sporadic nature of the disease; in future, the importance of genetic diagnostic tools will grow. Currently, the analysis of individual genes, such as *OTOF*, *MPZ* and *OPA1*, remains essential, but next generation sequencing enables testing for mutations in a large array of relevant genes and will support clinical diagnostics of hearing impairment disorders in near future¹²⁹. Strong bioinformatical analysis methods and validation by Sanger sequencing are required to identify pathogenicity of putative mutations.

Table 1 | A brief guide to clinical investigation of auditory neuropathy

Newborn/prelingual infants	Postlingual children and adults
Primary analysis	
Otoacoustic emissions (DPOAE/TEOAE)	Otoacoustic emissions (DPOAE/TEOAE)
Tympanometry (1000 Hz)	Tympanometry (1000 Hz in children aged <6 years; otherwise 226 Hz)
Acoustic reflex	Acoustic reflex
ABRs:	ABRs:
Separate testing of rarefaction and condensation clicks	Separate testing of rarefaction and condensation clicks
ABR peak amplitudes and latencies	ABR peak amplitudes and latencies
Infant reflex audiometry	Pure tone audiometry (<6 years: play audiometry)
Play audiometry	Speech audiometry (also in background noise)
Advanced analysis	
Electrocochleography	Electrocochleography
Promontory electrical stimulation: electrically evoked ABRs	Promontory electrical stimulation: electrically evoked ABRs (children)
Contralateral suppression of OAE (optional)	Contralateral suppression of OAE (optional)
MRI scan	MRI scan
Detailed psychophysical testing not feasible	Detailed psychophysics: tone decay, gap detection, frequency discrimination
Acoustically evoked potentials with middle/long latency (optional)	Acoustically evoked potentials with middle/long latency (optional)
Detailed psychophysical testing not feasible	Speech and language
Vestibular function (as feasible)	Vestibular function (videoculography, VEMP, vHIT)
Neurological assessment	Neurological assessment
Ophthalmological assessment	Ophthalmological assessment
Genetic sequencing	Genetic sequencing
Evaluation of hearing aids/cochlear implant rehabilitation outcome	Evaluation of hearing aids/cochlear implant rehabilitation outcome

ABR, auditory brain stem response; DPOAE, distortion product otoacoustic emission; TEOAE, transient evoked otoacoustic emission; VEMP, vestibular evoked myogenic potential; vHIT, video Head Impulse Test.

Current options for hearing rehabilitation

Counselling

Sufficient counselling is of critical importance for individuals affected by auditory synaptopathy or neuropathy, as well as for their families. The common but counterintuitive experience that patients ‘can hear but not understand’ (REF. 2) requires the doctor to explain the underlying pathophysiology of sound coding and neural signal propagation. Families need to understand that a synaptic or neural disorder of the peripheral auditory system can cause this type of hearing impairment. Neurological analysis and MRI can comfort patients and their families if providing evidence against a generalized neuropsychiatric disorder.

Cochlear implants versus hearing aids

We recommend fitting of hearing aids as the first-line therapy, even though they might not provide sufficient rehabilitation (see below and also discussion by Rance and Starr⁸⁷). In disorders such as auditory synaptopathy, in which spike generation and propagation are maintained, electrical stimulation of SGNs by cochlear implants is expected to provide efficient hearing rehabilitation. Cochlear implants have also proven helpful in auditory neuropathies arising

from SGN disease, such as those caused by mutations in the *OPA1* gene, probably because the strong electrical stimuli achieve temporally well-defined SGN activation.

Considerations regarding young infants

Rehabilitation and counselling can be particularly challenging in young infants. Balancing diagnostic certainty and best chances for rehabilitation with cochlear implants in this patient group is particularly important, but also particularly challenging: certainty about the disorder increases with more reliable psychophysics as development progresses, repeated physiological testing (potentially showing maturation of neural responses such as ABRs) and the observed outcome from early hearing aid rehabilitation; on the other hand, the outcome from cochlear implant rehabilitation, which is currently considered as a good option for subjects with auditory synaptopathy or neuropathy^{130,131}, is better the earlier the implantation is performed.

Uncertainties in outcomes

Unfortunately, the data on the outcome of auditory neuropathy rehabilitation are limited¹³². At first glance, solely

amplifying sound is not expected to improve auditory signalling, whereas the strong electrical stimulus of a cochlear implant can synchronously activate SGNs even in cases of impaired auditory nerve function¹³³. However, although the outcome of auditory neuropathy in children with cochlear implants can indeed be similar to those with hearing impairment owing to other aetiologies, it does not seem to be substantially better than that of auditory neuropathy fitted with hearing aids^{134,135}. Of course, it is quite possible that the patients with auditory neuropathy who were fitted with cochlear implants were more severely affected and would have had a worse outcome with hearing aids. Moreover, experimental animal studies demonstrated a clear relationship between the amplitude of eABRs and the number of remaining SGNs^{136,137}. In addition, mice with peripheral myelination deficits exhibit prolonged eABR latencies, elevated eABR thresholds and diminished eABR amplitudes¹³⁸. The uncertainties in outcome with cochlear implant rehabilitation need to be considered for auditory neuropathy patients with neural aetiologies, whereas cochlear implant rehabilitation can generally be recommended for patients with severe auditory synaptopathy¹³⁹. Therefore, an improved ability to accurately localize the site of lesion, using a combination of genetic diagnostics and transtympanic electrocochleography, would be of great benefit when considering cochlear implant rehabilitation.

Future perspectives for rehabilitation

Progress in hearing aid and cochlear implant technology will, hopefully, improve the rehabilitation of auditory neuropathy. Specifically, the use of directional microphones and smart signal processing strategies can partially restore or improve speech comprehension. In the longer run, optogenetic stimulation of the auditory nerve¹⁴⁰ promises improved frequency resolution for coding with cochlear implants¹⁴¹.

Recently, preclinical studies have indicated that gene therapy might become a feasible cure of specific forms of auditory neuropathy. Mouse studies have demonstrated successful restoration of hearing by virus-mediated gene replacement in experimental genetic auditory

synaptopathy^{18,142}, and viral vectors are currently being optimized for efficient and specific gene transfer. The size of some of the genes of interest, such as *OTOF1* and genes encoding $\text{Ca}_v1.3$ Ca^{2+} channels, exceeds the current packaging capacity of the most-efficient and non-pathogenic viral vector, the adeno-associated virus. Procedures for inoculation of the virus into the cochlea have been developed in various species and are now already used in a clinical trial aiming to *trans*-differentiate supporting cells into hair cells¹⁴³. A recent mouse study demonstrated that viral expression of nerve growth factor in the cochlea restored hair cell synapses following noise trauma, and mitigated noise-induced hearing loss¹²³. Although the results of all these efforts are promising, we anticipate that provision of these therapies to human patients with auditory synaptopathy or neuropathy is still a decade away.

Conclusions

Auditory neuropathy is a nosological term that was coined for a hearing impairment that commences downstream from mechanoelectrical transduction and cochlear amplification of OHCs. Human genetics and analysis of animal models of auditory neuropathy have jointly elucidated disease mechanisms that include loss of IHCs or IHC ribbon synapses, impaired synaptic transmission to SGNs and disrupted propagation of auditory information along the auditory nerve. Disorders of IHC ribbon synapses (auditory synaptopathy) arise from genetic defects as well as from sound overexposure. Deep phenotyping involving audiology, neurology, ophthalmology and genetics enables diagnosis of auditory neuropathy and, in some cases, identification of sensory, synaptic or neural disease mechanisms. Rehabilitation of hearing mostly relies on hearing aids (beneficial in some cases) and cochlear implants, which are particularly beneficial for individuals with severe auditory synaptopathy. Finally, future therapeutic options to restore hearing include gene replacement therapy for selected genetic disorders, hair cell regeneration, improved cochlear implants and local application of neurotrophins to, for example, improve synaptogenesis following acoustic synaptic insult.

- World Health Organization. Primary ear and hearing care training resource. Advanced level. [online], http://www.who.int/pbd/deafness/activities/hearing_care/advanced.pdf (2006).
- Starr, A., Picton, T. W., Siner, Y., Hood, L. J. & Berlin, C. I. Auditory neuropathy. *Brain* **119**, 741–754 (1996).
Starr and colleagues first coined the term 'auditory neuropathy' and provided a detailed auditory phenotype for hereditary sensory and motor neuropathy.
- Zeng, F.-G., Kong, Y.-Y., Michalewski, H. J. & Starr, A. Perceptual consequences of disrupted auditory nerve activity. *J. Neurophysiol.* **93**, 3050–3063 (2005).
- Moser, T. *et al.* Diagnostik und therapie der auditorischen synaptopathie/neuropathie. *HNO* **54**, 833–841 (in German) (2006).
- Sutton, G. J. *et al.* Assessment and management of auditory neuropathy/auditory dys-synchrony: a recommended protocol. *Newborn Hearing Screening Programme England* [online], <https://www.escholar.manchester.ac.uk/uk-ac-man-scw:1d33279> (2004).
- Starr, A., Zeng, F. G., Michalewski, H. J. & Moser, T. in *The Senses: A Comprehensive Reference Vol 3. Ch. 23* (eds Basbaum, A. I. *et al.*) 397–412 (Academic Press, 2008).
- Penido, R. C. & Isaac, M. L. Prevalence of auditory neuropathy spectrum disorder in an auditory health care service. *Braz. J. Otorhinolaryngol.* **79**, 429–433 (2013).
- Rance, G. *et al.* Clinical findings for a group of infants and young children with auditory neuropathy. *Ear Hear.* **20**, 238–252 (1999).
- Foerst, A. *et al.* Prevalence of auditory neuropathy/synaptopathy in a population of children with profound hearing loss. *Int. J. Pediatr. Otorhinolaryngol.* **70**, 1415–1422 (2006).
- Rodríguez-Ballesteros, M. *et al.* A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (*OTOF*) in subjects with nonsyndromic hearing impairment and auditory neuropathy. *Hum. Mutat.* **29**, 823–831 (2008).
- Thirlwall, A. S., Brown, D. J., McMillan, P. M., Barker, S. E. & Lesperance, M. M. Phenotypic characterization of hereditary hearing impairment linked to DFNA25. *Arch. Otolaryngol. Head Neck Surg.* **129**, 830–835 (2003).
- Matthews, G. & Fuchs, P. The diverse roles of ribbon synapses in sensory neurotransmission. *Nat. Rev. Neurosci.* **11**, 812–822 (2010).
- Bech-Hansen, N. T. *et al.* Loss-of-function mutations in a calcium-channel $\alpha 1$ -subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nat. Genet.* **19**, 264–267 (1998).
- Strom, T. M. *et al.* An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nat. Genet.* **19**, 260–263 (1998).
- Zeit, C. *et al.* Mutations in *CABP4*, the gene encoding the Ca^{2+} -binding protein 4, cause autosomal recessive night blindness. *Am. J. Hum. Genet.* **79**, 657–667 (2006).
- Khimich, D. *et al.* Hair cell synaptic ribbons are essential for synchronous auditory signalling. *Nature* **434**, 889–894 (2005).
Using mouse mutants with inner hair cell synapses deficient in the scaffold protein Bassoon and the synaptic ribbon, Khimich *et al.* demonstrated how high rates of presynaptic vesicle exocytosis are required for synchronous activation of the spiral ganglion neurons, reflected by the compound action potential.

17. Buran, B. N. *et al.* Onset coding is degraded in auditory nerve fibers from mutant mice lacking synaptic ribbons. *J. Neurosci.* **30**, 7587–7597 (2010).
18. Jung, S. *et al.* Disruption of adaptor protein 2 (AP-2) in cochlear hair cells impairs vesicle reloading of synaptic release sites and hearing. *EMBO J.* **34**, 2686–2702 (2015).
Near-complete restoration of hearing in a knockout mouse model of auditory synaptopathy by a postnatal gene transfer via injection of a viral vector into the cochlea.
19. Pangrsic, T. *et al.* Hearing requires otoferlin-dependent efficient replenishment of synaptic vesicles in hair cells. *Nat. Neurosci.* **13**, 869–876 (2010).
This study demonstrated that reduced otoferlin levels disrupt vesicle replenishment and, therefore, impair indefatigable transmitter release from inner hair cells.
20. Parkinson, N. J. *et al.* Mutant β -spectrin 4 causes auditory and motor neuropathies in quivering mice. *Nat. Genet.* **29**, 61–65 (2001).
21. Lacas-Gervais, S. *et al.* β IVS1 spectrin stabilizes the nodes of Ranvier and axon initial segments. *J. Cell Biol.* **166**, 983–990 (2004).
22. Wichmann, C. & Moser, T. Relating structure and function of inner hair cell ribbon synapses. *Cell Tissue Res.* **361**, 95–114 (2015).
23. Fuchs, P. A. Time and intensity coding at the hair cell's ribbon synapse. *J. Physiol.* **566**, 7–12 (2005).
24. Moser, T., Neef, A. & Khimich, D. Mechanisms underlying the temporal precision of sound coding at the inner hair cell ribbon synapse. *J. Physiol.* **576**, 55–62 (2006).
25. Pangršič, T., Reisinger, E. & Moser, T. Otoferlin: a multi-C₂ domain protein essential for hearing. *Trends Neurosci.* **35**, 671–680 (2012).
A review of the molecular physiology of inner hair cell ribbon synapses, with a focus on otoferlin.
26. Nouvian, R. *et al.* Exocytosis at the hair cell ribbon synapse apparently operates without neuronal SNARE proteins. *Nat. Neurosci.* **14**, 411–413 (2011).
27. Strenzke, N. *et al.* Complexin-I is required for high-fidelity transmission at the endbulb of held auditory synapse. *J. Neurosci.* **29**, 7991–8004 (2009).
28. Uthaiiah, R. C. & Hudspeth, A. J. Molecular anatomy of the hair cell's ribbon synapse. *J. Neurosci.* **30**, 12387–12399 (2010).
29. Vogl, C. *et al.* Unconventional molecular regulation of synaptic vesicle replenishment in cochlear inner hair cells. *J. Cell. Sci.* **128**, 638–644 (2015).
30. Roux, I. *et al.* Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* **127**, 277–289 (2006).
This study describes the auditory phenotype of otoferlin knockout mice and demonstrates that otoferlin has an essential role in inner hair cell exocytosis.
31. Seal, R. P. *et al.* Sensorineural deafness and seizures in mice lacking vesicular glutamate transporter 3. *Neuron* **57**, 263–275 (2008).
This *Slc17A8*-knockout mouse study demonstrated that VGLUT3 has an essential role in sound encoding at the inner hair cell ribbon synapse.
32. Ruel, J. *et al.* Impairment of *SLC17A8* encoding vesicular glutamate transporter-3, VGLUT3, underlies nonsyndromic deafness DFNA25 and inner hair cell dysfunction in null mice. *Am. J. Hum. Genet.* **83**, 278–292 (2008).
This study revealed that a mutation in *Vglut3* underlies autosomal dominant deafness-25 and showed the requirement of VGLUT3 in sound encoding at the inner hair cell ribbon synapse.
33. Platzter, J. *et al.* Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca²⁺ channels. *Cell* **102**, 89–97 (2000).
34. Brandt, A., Striessnig, J. & Moser, T. Ca_v1.3 channels are essential for development and presynaptic activity of cochlear inner hair cells. *J. Neurosci.* **23**, 10832–10840 (2003).
35. Geisler, C. D. *From Sound to Synapse: Physiology of the Mammalian Ear* (Oxford Univ. Press, 1998).
36. Rutherford, M. A. & Moser, T. In *The Primary Auditory Neurons of the Mammalian Cochlea* (eds Dabdoub, A. *et al.*) 117–156 (Springer-Verlag, 2016).
This book chapter is part of a recently published textbook on spiral ganglion neurons, and provides a comprehensive overview on the inner hair cell–spiral ganglion neuron synapse.
37. Mo, Z. L., Adamson, C. L. & Davis, R. L. Dendrotoxin-sensitive K⁺ currents contribute to accommodation in murine spiral ganglion neurons. *J. Physiol.* **542**, 763 (2002).
38. Rutherford, M. A., Chapochnikov, N. M. & Moser, T. Spike encoding of neurotransmitter release timing by spiral ganglion neurons of the cochlea. *J. Neurosci.* **32**, 4775–4789 (2012).
39. Glowatzki, E. & Fuchs, P. A. Transmitter release at the hair cell ribbon synapse. *Nat. Neurosci.* **5**, 147–154 (2002).
The first postsynaptic patch-clamp recording from the inner hair cell ribbon synapse of the rat demonstrates massive heterogeneity in size and shape of the excitatory postsynaptic currents, interpreted to reflect synchronous release of multiple vesicles despite the absence of a presynaptic action potential.
40. Chapochnikov, N. M. *et al.* Uniquantal release through a dynamic fusion pore is a candidate mechanism of hair cell exocytosis. *Neuron* **17**, 1389–1403 (2014).
41. Hossain, W. A., Antic, S. D., Yang, Y., Rasband, M. N. & Morest, D. K. Where is the spike generator of the cochlear nerve? Voltage-gated sodium channels in the mouse cochlea. *J. Neurosci.* **25**, 6857–6868 (2005).
42. Yasunaga, S. *et al.* A mutation in *OTOF*, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. *Nat. Genet.* **21**, 363–369 (1999).
43. Varga, R. *et al.* OTOF mutations revealed by genetic analysis of hearing loss families including a potential temperature sensitive auditory neuropathy allele. *J. Med. Genet.* **43**, 576–581 (2006).
44. Marlin, S. *et al.* Temperature-sensitive auditory neuropathy associated with an otoferlin mutation: deafening fever! *Biochem. Biophys. Res. Commun.* **394**, 737–742 (2010).
45. Wang, D.-Y. *et al.* Screening mutations of *OTOF* gene in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy. *BMC Med. Genet.* **11**, 79 (2010).
46. Romanos, J. *et al.* Novel *OTOF* mutations in Brazilian patients with auditory neuropathy. *J. Hum. Genet.* **54**, 382–385 (2009).
47. Matsunaga, T. *et al.* A prevalent founder mutation and genotype–phenotype correlations of *OTOF* in Japanese patients with auditory neuropathy. *Clin. Genet.* **82**, 425–432 (2012).
48. McNeil, P. L. & Kirchhausen, T. An emergency response team for membrane repair. *Nat. Rev. Mol. Cell. Biol.* **6**, 499–505 (2005).
49. Liu, J. *et al.* Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nat. Genet.* **20**, 31–36 (1998).
50. Bansal, D. *et al.* Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* **423**, 168–172 (2003).
51. Jiménez, J. L. & Bashir, R. *In silico* functional and structural characterisation of ferlin proteins by mapping disease-causing mutations and evolutionary information onto three-dimensional models of their C2 domains. *J. Neurol. Sci.* **260**, 114–123 (2007).
52. Johnson, C. P. & Chapman, E. R. Otoferlin is a calcium sensor that directly regulates SNARE-mediated membrane fusion. *J. Cell Biol.* **191**, 187–197 (2010).
53. Padmanarayana, M. *et al.* Characterization of the lipid binding properties of Otoferlin reveals specific interactions between PI(4,5)P₂ and the C2C and C2F domains. *Biochemistry* **53**, 5023–5033 (2014).
54. Ramakrishnan, N. A., Drescher, M. J. & Drescher, D. G. Direct interaction of otoferlin with syntaxin 1A, SNAP-25, and the L-type voltage-gated calcium channel Cav1.3. *J. Biol. Chem.* **284**, 1364–1372 (2009).
55. Helfmann, S. *et al.* The crystal structure of the C₂A domain of otoferlin reveals an unconventional top loop region. *J. Mol. Biol.* **406**, 479–490 (2011).
56. Fuson, K. *et al.* Alternate splicing of dysferlin C2A confers Ca²⁺-dependent and Ca²⁺-independent binding for membrane repair. *Structure* **22**, 104–115 (2014).
57. Santarelli, R., del Castillo, I., Cama, E., Scimemi, P. & Starr, A. Audibility, speech perception and processing of temporal cues in ribbon synaptic disorders due to *OTOF* mutations. *Hear. Res.* **330** (Pt B), 200–212 (2015).
58. Reisinger, E. *et al.* Probing the functional equivalence of otoferlin and synaptotagmin 1 in exocytosis. *J. Neurosci.* **31**, 4886–4895 (2011).
59. Dulon, D., Safieddine, S., Jones, S. M. & Petit, C. Otoferlin is critical for a highly sensitive and linear calcium-dependent exocytosis at vestibular hair cell ribbon synapses. *J. Neurosci.* **29**, 10474–10487 (2009).
60. Varga, R. *et al.* Non-syndromic recessive auditory neuropathy is the result of mutations in the otoferlin (*OTOF*) gene. *J. Med. Genet.* **40**, 45–50 (2003).
61. Schwander, M. *et al.* A forward genetics screen in mice identifies recessive deafness traits and reveals that pejkakin is essential for outer hair cell function. *J. Neurosci.* **27**, 2163–2175 (2007).
62. Wynne, D. P. *et al.* Loudness adaptation accompanying ribbon synapse and auditory nerve disorders. *Brain* **136**, 1626–1638 (2013).
63. Duncker, S. V. *et al.* Otoferlin couples to clathrin-mediated endocytosis in mature cochlear inner hair cells. *J. Neurosci.* **33**, 9508–9519 (2013).
64. Greene, C. C. *et al.* DFNA25, a novel locus for dominant nonsyndromic hereditary hearing impairment, maps to 12q21–24. *Am. J. Hum. Genet.* **68**, 254–260 (2001).
65. Obholzer, N. *et al.* Vesicular glutamate transporter 3 is required for synaptic transmission in zebrafish hair cells. *J. Neurosci.* **28**, 2110–2118 (2008).
66. Petek, E. *et al.* Molecular characterization of a 12q22–q24 deletion associated with congenital deafness: confirmation and refinement of the DFNA25 locus. *Am. J. Med. Genet. A* **117A**, 122–126 (2003).
67. Baig, S. M. *et al.* Loss of Ca_v1.3 (*CACNA1D*) function in a human channelopathy with bradycardia and congenital deafness. *Nat. Neurosci.* **14**, 77–84 (2011).
The first report of a human deafness syndrome attributed to a loss-of-function mutation in *CACNA1D*.
68. Surmeier, D. J. Calcium, ageing, and neuronal vulnerability in Parkinson's disease. *Lancet Neurol.* **6**, 933–938 (2007).
69. McKinney, B. C. & Murphy, G. G. The L-Type voltage-gated calcium channel Ca_v1.3 mediates consolidation, but not extinction, of contextually conditioned fear in mice. *Learn. Mem.* **13**, 584–589 (2006).
70. Neef, J. *et al.* The Ca²⁺ channel subunit β 2 regulates Ca²⁺ channel abundance and function in inner hair cells and is required for hearing. *J. Neurosci.* **29**, 10730 (2009).
71. Wycisk, K. A. *et al.* Mutation in the auxiliary calcium-channel subunit *CACNA2D4* causes autosomal recessive cone dystrophy. *Am. J. Hum. Genet.* **79**, 973–977 (2006).
72. Schrauwen, I. *et al.* A mutation in *CABP2*, expressed in cochlear hair cells, causes autosomal-recessive hearing impairment. *Am. J. Hum. Genet.* **91**, 636–645 (2012).
A report of a hearing impairment in a family with a *CABP2* mutation, suggesting that inner hair cell synaptic dysfunction is caused by impaired presynaptic Ca²⁺ influx.
73. Haeseleer, F. *et al.* Essential role of Ca²⁺-binding protein 4, a Ca_v1.4 channel regulator, in photoreceptor synaptic function. *Nat. Neurosci.* **7**, 1079–1087 (2004).
74. Santarelli, R. *et al.* *OPA1*-related auditory neuropathy: site of lesion and outcome of cochlear implantation. *Brain* **138**, 563–576 (2015).
75. Yu-Wai-Man, P. *et al.* Multi-system neurological disease is common in patients with *OPA1* mutations. *Brain* **133**, 771–786 (2010).
76. La Morgia, C., Carbonelli, M., Barboni, P., Sadun, A. A. & Carelli, V. Medical management of hereditary optic neuropathies. *Front. Neurol.* **5**, 141 (2014).
77. Alexander, C. *et al.* *OPA1*, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat. Genet.* **26**, 211–215 (2000).
78. Delettre, C. *et al.* Nuclear gene *OPA1*, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat. Genet.* **26**, 207–210 (2000).
79. Kasahara, A. & Scorrano, L. Mitochondria: from cell death executioners to regulators of cell differentiation. *Trends Cell Biol.* **24**, 761–770 (2014).
80. Ferré, M. *et al.* Molecular screening of 980 cases of suspected hereditary optic neuropathy with a report on 77 novel *OPA1* mutations. *Hum. Mutat.* **30**, E692–E705 (2009).
81. Alavi, M. V. *et al.* A splice site mutation in the murine *Opa1* gene features pathology of autosomal dominant optic atrophy. *Brain* **130**, 1029–1042 (2006).
82. Starr, A., Dong, C. J. & Michalewski, H. J. Brain potentials before and during memory scanning. *Electroencephalogr. Clin. Neurophysiol.* **99**, 28–37 (1996).
83. Starr, A. Pathology and physiology of auditory neuropathy with a novel mutation in the *MPZ* gene (Tyr145→Ser). *Brain* **126**, 1604–1619 (2003).

84. Kabzińska, D. *et al.* Late-onset Charcot-Marie-Tooth type 2 disease with hearing impairment associated with a novel Pro105Thr mutation in the *MPZ* gene. *Am. J. Med. Genet. A* **143A**, 2196–2199 (2007).
85. Verhagen, W. I. M. *et al.* Sensorineural hearing impairment in patients with Pmp22 duplication, deletion, and frameshift mutations. *Otol. Neurotol.* **26**, 405–414 (2005).
86. Kovach, M. J. *et al.* Anticipation in a unique family with Charcot-Marie-Tooth syndrome and deafness: delineation of the clinical features and review of the literature. *Am. J. Med. Genet.* **108**, 295–303 (2002).
87. Rance, G. & Starr, A. Pathophysiological mechanisms and functional hearing consequences of auditory neuropathy. *Brain* **138**, 3141–3158 (2015).
In this review, Rance and Starr provide a comprehensive overview of spiral ganglion disorders and their clinical manifestations.
88. Delmaghani, S. *et al.* Mutations in the gene encoding peykin, a newly identified protein of the afferent auditory pathway, cause DFNB59 auditory neuropathy. *Nat. Genet.* **38**, 770–778 (2006).
89. Borck, G. *et al.* High frequency of autosomal-recessive DFNB59 hearing loss in an isolated Arab population in Israel. *Clin. Genet.* **82**, 271–276 (2012).
90. Ebermann, I. *et al.* Truncating mutation of the *DFNB59* gene causes cochlear hearing impairment and central vestibular dysfunction. *Hum. Mutat.* **28**, 571–577 (2007).
91. Hashemzadeh Chaleshtori, M. *et al.* Novel mutations in the peykin gene are associated with autosomal recessive non-syndromic hearing loss in Iranian families. *Clin. Genet.* **72**, 261–263 (2007).
92. Collin, R. W. J. *et al.* Involvement of *DFNB59* mutations in autosomal recessive nonsyndromic hearing impairment. *Hum. Mutat.* **28**, 718–723 (2007).
93. Delmaghani, S. *et al.* Hypervulnerability to sound exposure through impaired adaptive proliferation of peroxisomes. *Cell* **163**, 894–906 (2015).
This study demonstrated that genetic disruption of peykin increases vulnerability of hair cells and neurons to noise exposure, providing an interesting experimental model for hearing impairment as a multifactorial disease.
94. Kim, T. B. *et al.* A gene responsible for autosomal dominant auditory neuropathy (AUNA1) maps to 13q14–21. *J. Med. Genet.* **41**, 872 (2004).
95. Starr, A. *et al.* A dominantly inherited progressive deafness affecting distal auditory nerve and hair cells. *J. Assoc. Res. Otolaryngol.* **5**, 411–426 (2004).
96. Schoen, C. J. *et al.* Increased activity of *Diaphanous homolog 3* (*DIAPH3*)/*diaphanous* causes hearing defects in humans with auditory neuropathy and in *Drosophila*. *Proc. Natl Acad. Sci. USA* **107**, 13396–13401 (2010).
97. Schoen, C. J., Burmeister, M. & Lesperance, M. M. *Diaphanous homolog 3* (*Diap3*) overexpression causes progressive hearing loss and inner hair cell defects in a transgenic mouse model of human deafness. *PLoS ONE* **8**, e56520 (2013).
98. Rance, G. *et al.* Speech perception ability in individuals with Friedreich ataxia. *Brain* **131**, 2002–2012 (2008).
99. Kirkim, G., Serbetcioglu, B., Erdag, T. K. & Ceryan, K. The frequency of auditory neuropathy detected by universal newborn hearing screening program. *Int. J. Pediatr. Otorhinolaryngol.* **72**, 1461–1469 (2008).
100. Schulman-Galambos, C. & Galambos, R. Brain stem evoked response audiometry in newborn hearing screening. *Arch. Otolaryngol.* **105**, 86–90 (1979).
101. Oh, W. *et al.* Association between peak serum bilirubin and neurodevelopmental outcomes in extremely low birth weight infants. *Pediatrics* **112**, 773–779 (2003).
102. Olds, C. & Oghalai, J. S. Audiologic impairment associated with bilirubin-induced neurologic damage. *Semin. Fetal Neonatal Med.* **20**, 42–46 (2015).
103. Smith, C. M., Barnes, G. P., Jacobson, C. A. & Oelberg, D. G. Auditory brainstem response detects early bilirubin neurotoxicity at low indirect bilirubin values. *J. Perinatol.* **24**, 730–732 (2004).
104. Amatu, M. G. *et al.* Selective inner hair cell loss in premature infants and cochlear pathological patterns from neonatal intensive care unit autopsies. *Arch. Otolaryngol. Head Neck Surg.* **127**, 629–636 (2001).
105. Uziel, A., Marot, M. & Pujol, R. The Gunn rat: an experimental model for central deafness. *Acta Otolaryngol.* **95**, 651–656 (1983).
106. Hauste, M. D. *et al.* Acute hyperbilirubinaemia induces presynaptic neurodegeneration at a central glutamatergic synapse. *J. Physiol.* **588**, 4683–4693 (2010).
107. Spencer, R. F., Shaia, W. T., Gleason, A. T., Sismanis, A. & Shapiro, S. M. Changes in calcium-binding protein expression in the auditory brainstem nuclei of the jaundiced Gunn rat. *Hear. Res.* **171**, 129–141 (2002).
108. Attias, J., Raveh, E., Aizer-Dannon, A., Bloch-Mimouni, A. & Fattal-Valevski, A. Auditory system dysfunction due to infantile thiamine deficiency: long-term auditory sequelae. *Audiol. Neurotol.* **17**, 309–320 (2012).
109. Oishi, K. *et al.* Targeted disruption of *Slc19a2*, the gene encoding the high-affinity thiamine transporter Thtr-1, causes diabetes mellitus, sensorineural deafness and megaloblastosis in mice. *Hum. Mol. Genet.* **11**, 2951–2960 (2002).
110. Liberman, M. C., Tartaglino, E., Fleming, J. C. & Neufeld, E. J. Deletion of *SLC19A2*, the high affinity thiamine transporter, causes selective inner hair cell loss and an auditory neuropathy phenotype. *J. Assoc. Res. Otolaryngol.* **7**, 211–217 (2006).
111. Yang, C.-H., Schrepfer, T. & Schacht, J. Age-related hearing impairment and the triad of acquired hearing loss. *Front. Cell. Neurosci.* **9**, 276 (2015).
This article, part of a special review issue of Frontiers in Cellular Neuroscience, provides a detailed update on sensory hair cell death and studies aiming at inducing hair cell regeneration.
112. Henry, W. R. & Mulroy, M. J. Afferent synaptic changes in auditory hair cells during noise-induced temporary threshold shift. *Hear. Res.* **84**, 81–90 (1995).
113. Puel, J. L., Pujol, R., Ladrech, S. & Eybalin, M. α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid electrophysiological and neurotoxic effects in the guinea-pig cochlea. *Neuroscience* **45**, 63–72 (1991).
114. Puel, J. L., Ruel, J., Gervais d'Aldin, C. & Pujol, R. Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. *Neuroreport* **9**, 2109–2114 (1998).
115. Stamatakis, S., Francis, H. W., Lehar, M., May, B. J. & Ryugo, D. K. Synaptic alterations at inner hair cells precede spiral ganglion cell loss in aging C57BL/6J mice. *Hear. Res.* **221**, 104–118 (2006).
116. Kujawa, S. G. & Liberman, M. C. Adding insult to injury: cochlear nerve degeneration after 'temporary' noise-induced hearing loss. *J. Neurosci.* **29**, 14077 (2009).
This study shows that in mice, sound overexposure that only temporarily reduces audibility can cause a permanent loss of inner hair cell ribbon synapses and subsequent loss of spiral ganglion neurons.
117. Sergeyenko, Y., Lall, K., Liberman, M. C. & Kujawa, S. G. Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. *J. Neurosci.* **33**, 13686–13694 (2013).
118. Kujawa, S. G. & Liberman, M. C. Synaptopathy in the noise-exposed and aging cochlea: primary neural degeneration in acquired sensorineural hearing loss. *Hear. Res.* **330**(Pt B), 191–199 (2015).
This Review is a part of a review series on auditory synapses and synaptopathies and summarizes a large body of animal work on synaptic alterations in noise-induced and age-related hearing loss.
119. Hakuba, N., Koga, K., Gyo, K., Usami, S. I. & Tanaka, K. Exacerbation of noise-induced hearing loss in mice lacking the glutamate transporter GLAST. *J. Neurosci.* **20**, 8750–8753 (2000).
120. Meyer, A. C. *et al.* Tuning of synapse number, structure and function in the cochlea. *Nat. Neurosci.* **12**, 444–453 (2009).
121. Furman, A. C., Kujawa, S. G. & Liberman, M. C. Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. *J. Neurophysiol.* **110**, 577–586 (2013).
122. Bourien, J. *et al.* Contribution of auditory nerve fibers to compound action potential of the auditory nerve. *J. Neurophysiol.* **112**, 1025–1039 (2014).
123. Wan, G., Gómez-Casati, M. E., Gigliello, A. R., Liberman, M. C. & Corfas, G. Neurotrophin-3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. *eLife* **3**, e03564 (2014).
124. Schaeffe, R. & McAlpine, D. Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* **31**, 13452–13457 (2011).
125. Starr, A. *et al.* Absence of both auditory evoked potentials and auditory percepts dependent on timing cues. *Brain* **114**, 1157–1180 (1991).
126. Starr, A. *et al.* Cochlear receptor (microphonic and summating potentials, otoacoustic emissions) and auditory pathway (auditory brain stem potentials) activity in auditory neuropathy. *Ear Hear.* **22**, 91 (2001).
127. Santarelli, R. *et al.* Abnormal cochlear potentials from deaf patients with mutations in the otoferlin gene. *J. Assoc. Res. Otolaryngol.* **10**, 545–556 (2009).
128. Pauli-Magnus, D. *et al.* Detection and differentiation of sensorineural hearing loss in mice using auditory steady-state responses and transient auditory brainstem responses. *Neuroscience* **149**, 673–684 (2007).
129. Shearer, A. E. & Smith, R. J. H. Massively parallel sequencing for genetic diagnosis of hearing loss: the new standard of care. *Otolaryngol. Head Neck Surg.* **153**, 175–182 (2015).
130. Dean, C., Felder, G. & Kim, A. H. Analysis of speech perception outcomes among patients receiving cochlear implants with auditory neuropathy spectrum disorder. *Otol. Neurotol.* **34**, 1610–1614 (2013).
131. Humphriss, R. *et al.* Does cochlear implantation improve speech recognition in children with auditory neuropathy spectrum disorder? A systematic review. *Int. J. Audiol.* **52**, 442–454 (2013).
132. Roush, P., Frymark, T., Venediktov, R. & Wang, B. Audiologic management of auditory neuropathy spectrum disorder in children: a systematic review of the literature. *Am. J. Audiol.* **20**, 159–170 (2011).
133. Giraudet, F. & Avan, P. Auditory neuropathies: understanding their pathogenesis to illuminate intervention strategies. *Curr. Opin. Neurol.* **25**, 50–56 (2012).
134. Rance, G. & Barker, E. J. Speech and language outcomes in children with auditory neuropathy/dys-synchrony managed with either cochlear implants or hearing aids. *Int. J. Audiol.* **48**, 313–320 (2009).
135. Ching, T. Y. C. *et al.* Impact of the presence of auditory neuropathy spectrum disorder (ANSD) on outcomes of children at three years of age. *Int. J. Audiol.* **52**, S55–S64 (2013).
136. Hall, R. D. Estimation of surviving spiral ganglion cells in the deaf rat using the electrically evoked auditory brainstem response. *Hear. Res.* **49**, 155–168 (1990).
137. Hall, R. D. Estimation of surviving spiral ganglion cells in the deaf rat using the electrically evoked auditory brainstem response. *Hear. Res.* **45**, 123–136 (1990).
138. Zhou, R., Abbas, P. J. & Assouline, J. G. Electrically evoked auditory brainstem response in peripherally myelin-deficient mice. *Hear. Res.* **88**, 98–106 (1995).
139. Rouillon, I. *et al.* Results of cochlear implantation in two children with mutations in the *OTOF* gene. *Int. J. Pediatr. Otorhinolaryngol.* **70**, 689–696 (2006).
140. Hernandez, V. H. *et al.* Optogenetic stimulation of the auditory pathway. *J. Clin. Invest.* **124**, 1114–1129 (2014).
141. Jeschke, M. & Moser, T. Considering optogenetic stimulation for cochlear implants. *Hear. Res.* **322**, 224–234 (2015).
142. Akil, O. *et al.* Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. *Neuron* **75**, 283–293 (2012).
This mouse study demonstrated a near-complete restoration of hearing in a knockout mouse model of auditory synaptopathy by a postnatal gene transfer via injection of a viral vector into the cochlea.
143. US National Library of Medicine. *ClinicalTrials.gov* [online] <https://clinicaltrials.gov/ct2/show/NCT02132130> (2015).

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Competing interests statement

The authors declare no competing interests.

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