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Review of Hair Cell Synapse Defects in Sensorineural Hearing Impairment

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Objective: To review new insights into the pathophysiology of sensorineural hearing impairment. Specifically, we address defects of the ribbon synapses between inner hair cells and spiral ganglion neurons that cause auditory synaptopathy.
Data Sources and Study Selection: Here, we review original publications on the genetics, animal models, and molecular mechanisms of hair cell ribbon synapses and their dysfunction.
Conclusion: Hair cell ribbon synapses are highly specialized to enable indefatigable sound encoding with utmost temporal precision. Their dysfunctions, which we term *auditory synaptopathies*, impair audibility of sounds to varying degrees but commonly affect neural encoding of acoustic temporal cues essential for speech comprehension. Clinical features of auditory synaptopathies are similar to those accompanying auditory neuropathy, a group of genetic and acquired disorders of spiral ganglion neurons. Genetic auditory synaptopathies include alterations of glutamate loading of synaptic vesicles, synaptic Ca^{2+} influx or synaptic vesicle turnover. Acquired synaptopathies include noise-induced hearing loss because of excitotoxic synaptic damage and subsequent gradual neural degeneration. Alterations of ribbon synapses likely also contribute to age-related hearing loss. **Key Words:** Genetics—Ion channel—Sensorineural hearing impairment—Sound coding—Synapses—Synaptopathy.

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Mechanisms of Sensorineural Hearing Impairment

Sensorineural hearing impairment encompasses various pathologies of the cochlea and auditory nerve. Based on human temporal bone histology Schuknecht and Igarashi (1) proposed a nosology for slowly progressing sensorineural hearing loss. They distinguished conditions affecting stria vascularis (disrupting cochlear ion homeostasis and energetics), organ of Corti (disrupting hair cell function), and neurons (disrupting transmission of auditory information to the brain). Recent advances in the identification of human deafness genes and their physiological characterization in mouse models have helped to elucidate specific cellular mechanisms contributing to sensory and neural hearing loss. Combining genetic, physiologic, and psychophysical approaches to human sensorineural hearing loss one aims to differentiate primary defects of cochlear ionic homeostasis and endolymph production, mechanoelectrical transduction at the hair bundle, electromechanical amplification of basilar membrane vibration by the electromotile outer hair cells, synaptic transmission at the inner hair cell synapses, and action potential generation and conduction by spiral ganglion neurons.

Figure 1A illustrates a physiology-based classification of sensorineural hearing loss. Transduction defects as well as disruption of cochlear ionic homeostasis and endolymph production cause a global dysfunction of the cochlea. For example, the most common hereditary deafness caused by mutations in the gene coding for Connexin 26 impair the endocochlear potential (2,3), which is a prerequisite for the function of hair cells. Defects of outer hair cell electromotility or loss of outer hair cells altogether disrupt cochlear amplification and present primarily with loss of audibility, abnormal loudness gain (recruitment), and impaired frequency discrimination (4,5). Otoacoustic emissions (OAEs), acoustic signals produced by outer hair cell amplification of sound-induced vibrations in the cochlea, are reduced or absent. However, suprathreshold stimuli still evoke synchronized neural potentials in auditory nerve and brainstem pathways identified as auditory brainstem responses (ABRs). These subjects typically have impairments of speech reception affecting mainly consonants and their performance benefit from hearing aids.

Disorders of inner hair cell synapses—auditory synaptopathies—cause evoked potentials of the early

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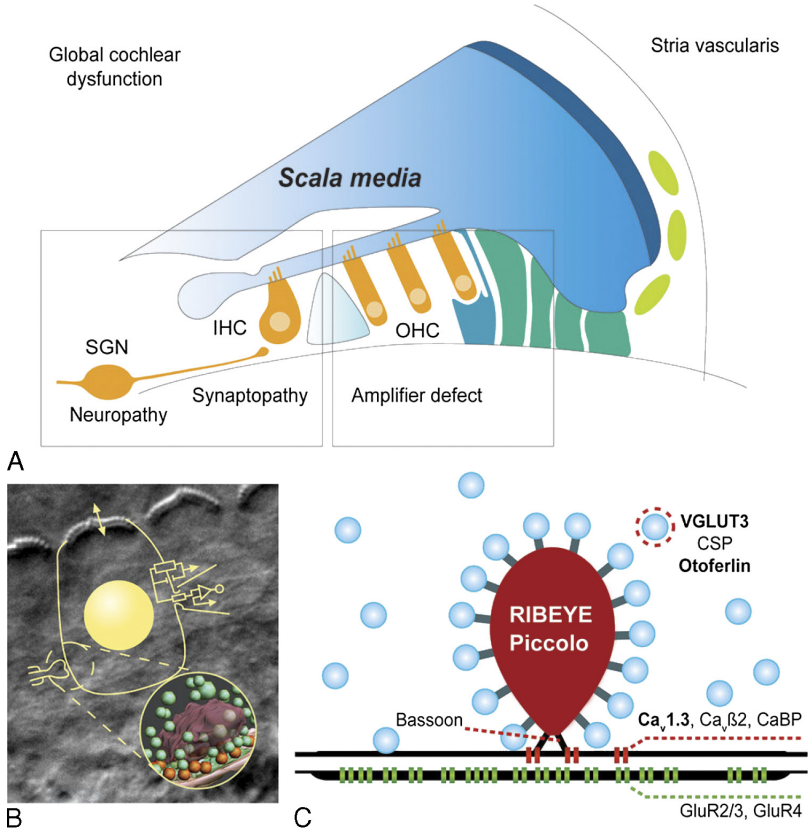

FIG. 1. Hair cell ribbon synapse—molecules affected in genetic auditory synaptopathies. *A*, Physiology-based classification of sensorineural hearing loss. Defects or loss of outer hair cells (OHC) disrupt cochlear amplification, defects or loss of inner hair cells (IHC) or their synapses disrupt synaptic encoding of sound, defects or loss of spiral ganglion neurons (SGN) disrupt encoding and/or conduction of auditory information. Defects of cochlear electrolyte homeostasis or mechanoelectrical transduction cause global dysfunction. *B*, Normaski image of the mouse organ of Corti with hair bundles of IHCs and schematic representation of a patch-clamped IHC and one of its ribbon synapses. Inset: model of a normal mouse IHC ribbon synapse obtained from electron tomography. *C*, Molecular anatomy of a normal mouse IHC ribbon synapse as derived from immunohistochemistry and molecular physiology. Otoferlin, VGLUT3, and $Ca_V1.3$ are the molecules so far identified to be the defect in human synaptopathy.

auditory pathway to be absent or abnormal (6,7). However, as cochlear amplification is functional, at least initially, OAEs and/or cochlear microphonic potentials are often present (7–10). Psychophysical findings in auditory synaptopathy vary from normal pure tone audiograms to complete deafness (6,8–14). Still, even when audibility is normal or minimally affected, speech comprehension is impaired and is often not improved by hearing aids (15). Defects of the auditory nerve (16) have similar findings as auditory synaptopathies rendering their differentiation difficult (15,16). Examination of temporal bones in subjects with neural disorders have shown both loss of auditory ganglion cells as well as demyelination of auditory nerve fibers (17). The effects of these changes are to seriously compromise the magnitude of auditory nerve activity, neural conduction speed, and to cause conduction block in affected fibers.

''Synaptopathy'' is a recently introduced term for a long-known nosological concept. Myasthenic disorders such as Myasthenia gravis and Lambert-Eaton syndrome are long established synaptopathies of the neuromuscular junction (18–21). Recently, synaptic dysfunction has received much attention as a potential disease mechanism in neuropsychiatric diseases such as Huntington's disease (22) and autism spectrum disorders (23,24). Although evidence indicates an important role of synaptic alterations in the pathophysiology of major brain diseases, their relevance as primary disease mechanism is an active topic of research (25).

Strong alterations of neuromuscular junction and synapses of the central nervous system are not compatible with life (e.g., ref. [26,27]). This is very different for ribbon synapses formed by sensory cells in the ear and retina. They are molecularly and structurally specialized and, to some extent, distinct from other synapses, such that mutations can specifically affect hearing and/or vision by impairing ribbon synapse function while sparing other synapses. The synaptic ribbon is an electron-dense structure that extends into the cytosol and tethers a halo of synaptic vesicles (Fig. 1B). Depending on the position of an inner hair cell along the tonotopic cochlear axis, it forms between 5 and 20 ribbon synapses (28) with the unbranched peripheral axons of spiral ganglion neurons (29). The exact role of this multi-protein nanomachinery is

subject of current studies (30–33). It is hypothesized to support a large pool of readily releasable vesicles and its replenishment after exocytosis. Its main molecular constituent is the protein Ribeye (34) (Fig. 1C) that is specific to ribbon synapses and thought to build the ribbon in a brick-stone like manner interacting with itself (35) and other proteins such as bassoon (36). Bassoon is a big scaffold protein (37), common to many synapses, and organizes the active zone of photoreceptors (38) and hair cells (30,33). While sharing some of the common scaffold proteins of the active zone, the hair cell ribbon synapse seems to otherwise employ different proteins than most other synapses (39–44) (Fig. 1C), some of which have been shown to be affected in hereditary synaptopathic hearing impairment.

GENETIC SYNAPTOPATHIES

Defects of Presynaptic Calcium Influx Into Inner Hair Cells

Unlike in other synapses, hair cell ribbon synapses use $Ca_V1.3$ L-type Ca^{2+} channels for stimulus-secretion coupling (45–47). Their active zones cluster tens of $Ca_V1.3$ L-type Ca^{2+} channels (33,48–52) (Fig. 2) that activate rapidly already at hyperpolarized potentials (53) and show only mild inactivation during ongoing stimulation (54,55). These functional properties arise from the unique molecular composition of the channel complex that involves interaction with numerous other proteins such as Ca^{2+} -binding proteins (56–58) (Fig. 2A). Recently, a loss of function mutation in the *CACNA1D* gene has been identified in a family with congenital deafness and bradycardia, signifying the importance of $Ca_V1.3$ for hearing and atrial pacemaking (12). Recently, a mutation of the *CABP2* gene has been demonstrated to cause a moderate sensorineural hearing impairment, which may be related to the lower potency of the mutant CaBP2 protein to inhibit calmodulin-mediated calcium dependent inactivation of the calcium current (57). Moreover, we note for a comparison that mutations in the genes coding for the poreforming α1F (*CACNA1F*) subunit (59,60) of the presynaptic $Ca_V1.4$ Ca^{2+} channels, the auxiliary α2δ4 subunit (61) and the interacting Ca^{2+} binding protein 4 (62) (*CaBP4*) cause human retinal disease such as night blindness probably by disturbing synaptic transmission at the photoreceptor ribbon synapses.

The human phenotype related to the loss of function *CACNA1D* mutation (12) is very closely resembled in *Cacna1d* knock-out mice, displaying both deafness and bradycardia (45,47). The mouse model allows for analysis of Ca^{2+} influx and the ensuing exocytosis in inner hair cells, which were both reduced by 90% (46) (Fig. 2C, D). This defect of hair cell transmitter release readily explains the lack of ABRs (Fig. 2E). The dramatic reduction of presensory and sensory afferent neural activity leads to

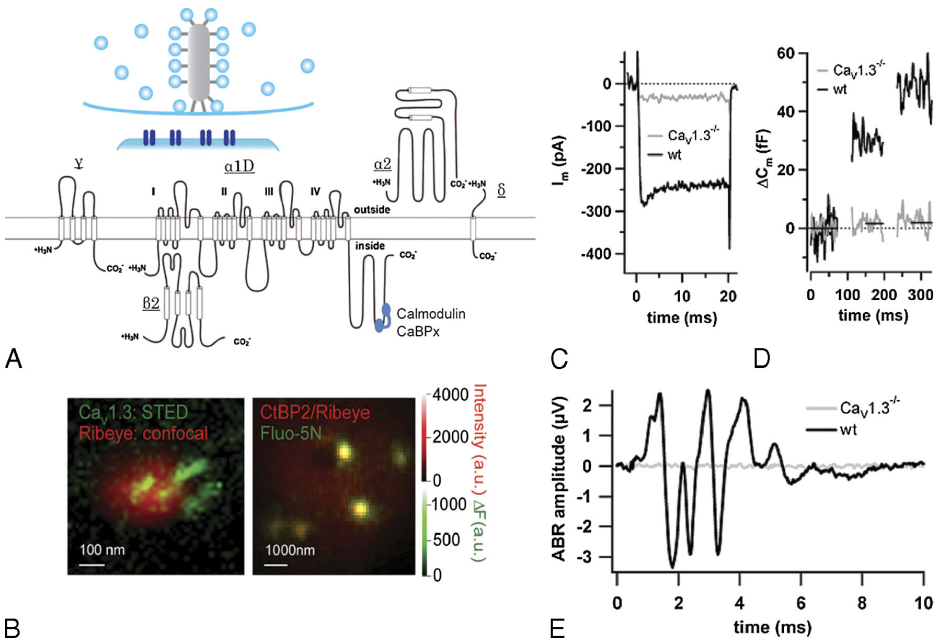

FIG. 2. Molecular physiology and pathology of hair cell calcium influx. *A*, Top: a defect in Ca^{2+} influx disrupts stimulus-secretion coupling, bottom: domain structures of the subunits forming the hair cell $Ca_V1.3$ Ca^{2+} channel: pore-forming α1D subunit, auxiliary ß2, α2δ, and γ subunits (adapted from Caterall, Pharmacol Rev 2005). *B*, Left: nanoanatomy of presynaptic $Ca_V1.3$ Ca^{2+} channel clusters resolved by STED microscopy after immunolabeling (taken from Frank et al., Neuron 2010); right: 5 presynaptic Ca^{2+} microdomains visualized as fluorescence hotspots of Fluo-5N indicator at the ribbon-occupied active zones (marked by a fluorescent Ribeye-binding peptide; taken from Frank et al., PNAS 2009). *C*, Representative Ca^{2+} currents and (*D*), membrane capacitance increments (ΔC_m, reflecting exocytic fusion of vesicles to the plasma membrane) of a normal IHC *(black)* and an IHC lacking the $Ca_V1.3$ Ca^{2+} channel *(gray)*: near-complete block of Ca^{2+} influx and exocytosis (taken from Brandt et al., 2003). *E*, Deafness of $Ca_V1.3$ deficient mice is indicated by lack of ABRs (representative recordings in response to 100 dB clicks).

substantial neurodevelopmental alterations in the auditory pathway (63–65) and to a progressive loss of hair cell afferent synapses, hair cells and spiral ganglion neurons (63,66), respectively. Interestingly, neither affected humans nor mice seem to have vestibular disorders. This is consistent with the finding of a sizable remaining Ca^{2+} current in vestibular hair cells of *Cacna1d* knock-out mice (47).

Genetic Alteration of Vesicular Glutamate Uptake in Hair Cells Disrupt Hearing

The glutamatergic ribbon synapses of hair cells use the transporter VGLUT3 to load their synaptic vesicles with glutamate (42,43,67), whereas all other glutamatergic synapses studied so far use VGLUT1 or 2 (68,69). Instead, in the CNS VGLUT3 is used by monaminergic and cholinergic neurons that co-release glutamate. Genetic ablation of Vglut3 function caused deafness in mice (42,43) and zebrafish (67) because of abolition of glutamate release (Fig. 3). Hair cell synapses remained surprisingly intact. They display robust Ca^{2+} influx and exocytosis of glutamate-devoid vesicles (43) (Fig. 3B), and spiral ganglion neurons exhibited robust postsynaptic receptor currents in response to application of exogenous glutamate (42). Loss of synapses, hair cells and spiral ganglion neurons proceeded at relatively slow pace (weeks rather than days as found for otoferlin mutants, see below) perhaps because of preserved release of trophic factors. Interestingly, no overt vestibular dysfunction was observed in *Vglut3* knock-out mice.

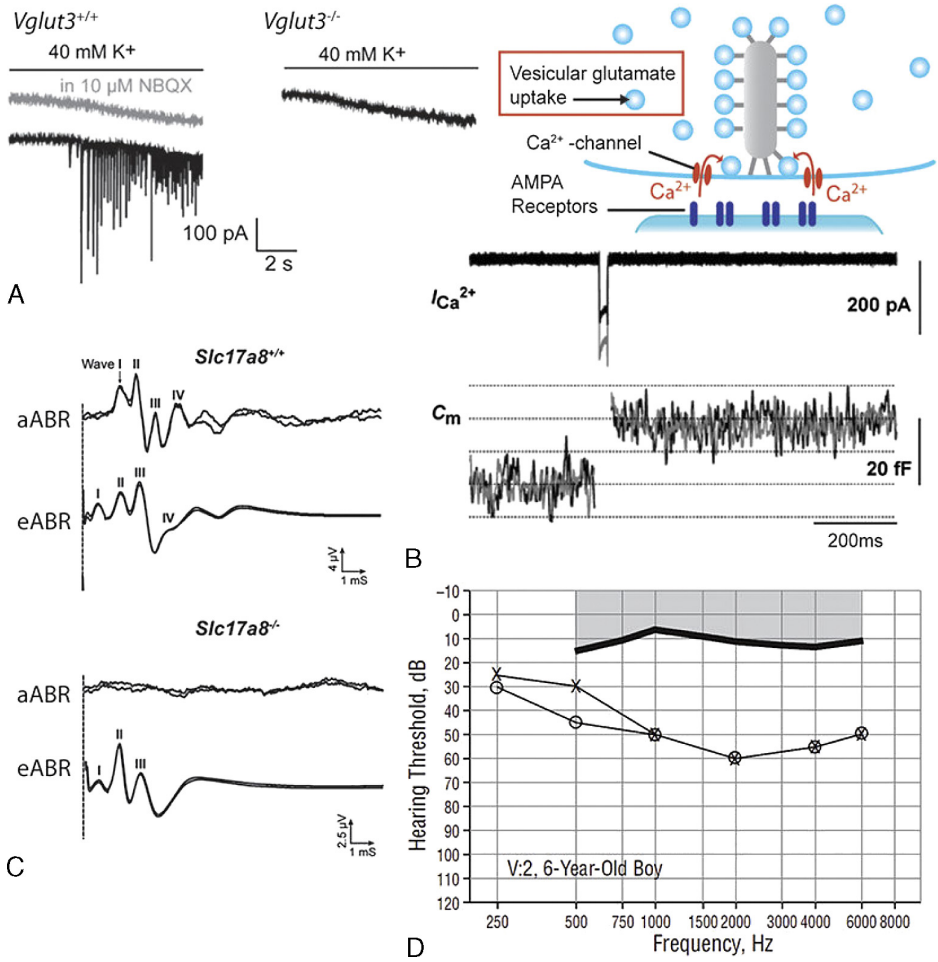

FIG. 3. VGLUT3-deficient hair cells lack glutamate release—human vglut3 mutations are responsible for progressive deafness DFNA25. *A*, Left: representative patch-clamp recording of excitatory postsynaptic currents (EPSCs) from a SGN terminal *(black)*, which are stimulated by superfusion with 40 mM K^+ and blocked by the AMPA receptor blocker NBQX *(gray)*; middle: lack of EPSCs in a representative recording from *Vglut3*-knockout mice (taken from Seal et al., Neuron 2008); right: genetic ablation Vglut3 function abolishes vesicular glutamate uptake and release. *B*, Despite ablation of *Vglut3* IHCs undergo Ca^{2+} influx (top panel) and exocytic fusion of vesicles (lower panel, taken from Ruel et al., Am J Hum Genet, 2008). Exocytosis of trophic factors potentially contributes to maintaining synaptic and neural integrity, such that degeneration proceeds more slowly than in $Ca_V1.3$ or otoferlin mutants. *C*, While both acoustically evoked ABR (aABR, top panel) and electrically evoked ABR (eABR, lower panel) are regularly recorded in control mice, only eABR but not aABR are observed in *Vglut3*-knockout mice (adapted from Ruel et al., *Am J Hum Genet*, 2008). *D*, pure tone audiogram of a DFNA25 affected boy displaying a moderate hearing impairment at 6 years of age (taken from Thirlwall et al., *Head Neck Surg* 2003).

First efforts toward virus-mediated transfer of *Vglut3* DNA into inner hair cells of *Vglut3* knock-out mice have yielded promising results: normal thresholds were restored for several weeks following viral injection into the cochlea (70). *Vglut3* knock-out mice and heterozygotes littermates showed EEG abnormalities indicative of a neocortical hyperexcitability, but myoclonic activity has not been detected (42). The human hearing impairment DFNA25

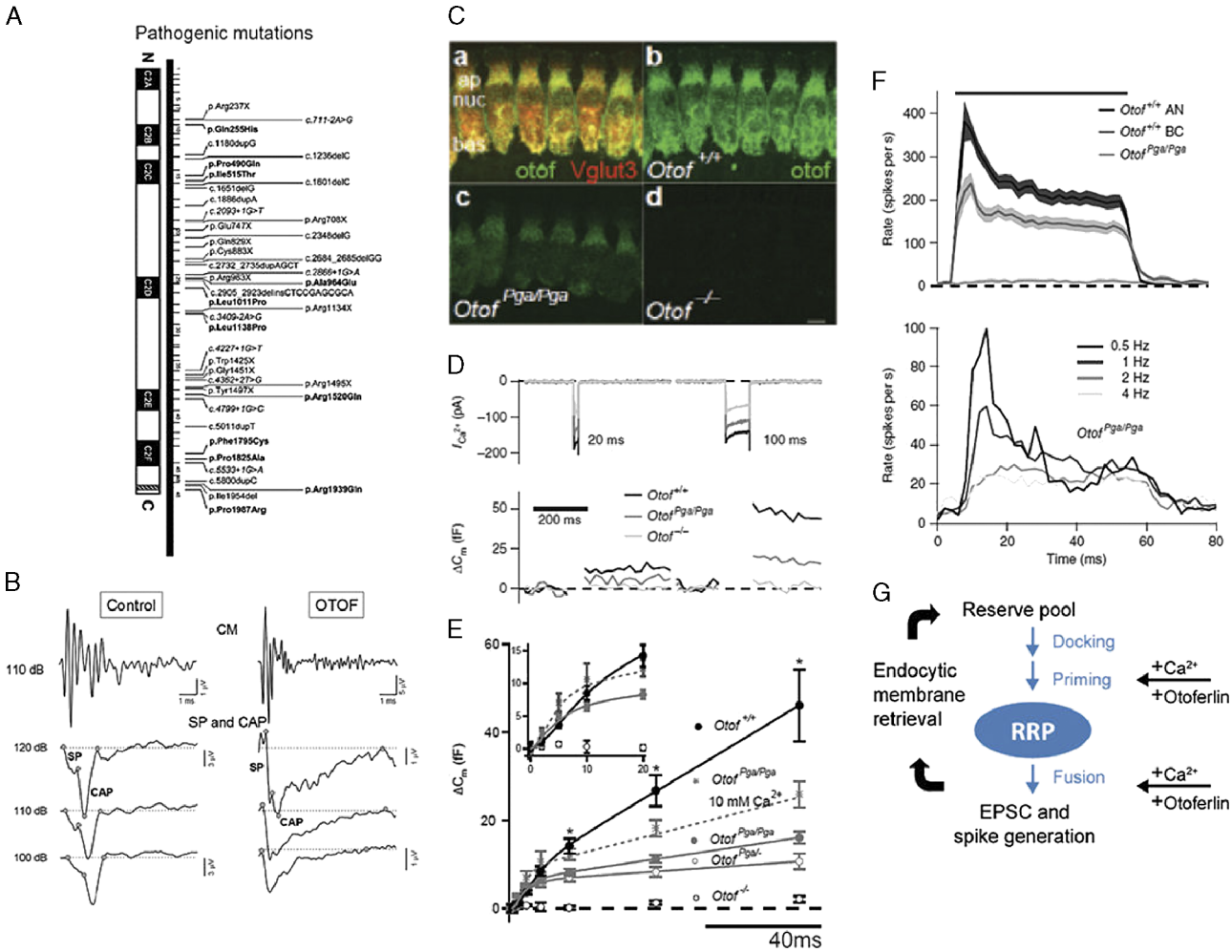

FIG. 4. OTOF mutations cause prelingual deafness DFNB9—otoferlin regulates replenishment and fusion of vesicles in IHCS. *A*, Domain structure of the multi-C_2-domain protein otoferlin (left) and amino acid changes caused by pathogenic mutations as published so far (right), modified from Rodriguez-Ballesteros et al., *Hum Mutat* 2003. *B*, Top: the CM is preserved in DFNB9, representative cochlear microphonic (CM) potentials of a control subject (left) and a deaf DFNB9 subject (right) in response to a 120-dB click stimulus. Lower: intact summating potentials (SPs) but strongly reduced compound action potentials (CAPs) in DFNB9, representative SP and CAP recorded from one control and DFNB9 subject in response to clicks of the designated sound pressure level. (modified from Santarelli et al., *JARO* 2009). *C*, (a), Projections of confocal images of IHC immunolabeled for otoferlin, which show a distribution similar to Vglut3. Reduced immunofluorescence in *Pachanga* mice (b, $Otof^{pga/pga}$) and lack of immunofluorescence in knock-out mice (c, $Otof^{-/-}$). *D*, Enhanced paired-pulse depression in a representative $Otof^{pga/pga}$ IHC assayed by measurements of exocytic membrane capacitance increments, indicating reduced recovery of the RRP from depletion (vesicle replenishment after stimulation). *E*, Exocytic membrane capacitance increments as a function of stimulation time for IHCs of normal mice ($Otof^{+/+}$), of $Otof^{pga/pga}$ mice (in elevated (10 mM) and normal [Ca^{2+}] in the bath), $Otof^{pga/-}$ mice (carrying only one *Pachanga* allele) and $Otof^{-/-}$ mice: progressive reduction of sustained exocytosis indicating reduced vesicle replenishment during ongoing stimulation (after 20 ms, i.e., exocytosis of the readily releasable pool (RRP). *F*, Poststimulus time histograms of extracellularly recorded spikes of auditory nerve fibers and principal cells of the cochlear nucleus of normal mice and $Otof^{pga/pga}$ mice (lumping all units together for the mutants) in response to tone burst stimulation 30 dB above threshold at 10 Hz: very low spike rates in $Otof^{pga/pga}$ mice. Reduction in stimulus rate restores an onset response in $Otof^{pga/pga}$ mice, which, however, is still lower than in control mice. This is consistent with strongly reduced vesicle replenishment limiting the RRP available for responses to transient stimuli. Spontaneous rate is less strongly reduced in $Otof^{pga/pga}$ mice. *G*, summary of otoferlin's roles in hair cell exocytosis: docking was found to be normal in all *Otof* mutants studied so far; therefore, defective replenishment is attributed to impaired vesicle priming. Vesicle replenishment does not suffice build-up of a standing RRP in the presence of substantial spontaneous release in vivo. In addition, a role of otoferlin in fusion is proposed. Panels *C*–*F* were taken from Pangrsic et al., *Nat Neurosci* 2010.

was first described in 2003 (13) and was then linked to a *VGLUT3* mutation in 2008 (43). Affected subjects become progressively hearing impaired starting during adolescence (Fig. 3D) and apparently lack other symptoms. Future studies are needed to address the precise mechanism of the progressive synaptopathy DFNA25.

Mutations in *OTOF* Cause Prelingual Deafness DFNB9 and Temperature-Sensitive Synaptic Hearing Impairment

Mutations in the *OTOF* gene coding for otoferlin—a member of the ferlin family of transmembrane multi-C_2-proteins (71,72), which is expressed in hair cells (73)—cause the prelingual deafness DFNB9 (6,8,10,74,75) and a temperature-sensitive hearing impairment (76,9,14) (Fig. 4). Since its identification, more than 50 pathogenic mutations of *OTOF* have been published (11) (Fig. 4A). Most mutations cause loss of otoferlin function and profound prelingual deafness (Fig. 4B), and affected individuals seem to benefit from cochlear implantation (77,78). Otoferlin is considered a synaptic vesicle protein because a direct association to synaptic vesicles was found by immuno-electron microscopy (73) and its distribution in hair cells is similar to that of the synaptic vesicle protein Vglut3 (79) (Fig. 4B). Ablation of *Otof* function in mice revealed a near complete abolition of hair cell exocytosis as the mechanism underlying DFNB9. Synapses were rapidly lost postnatally probably because of degeneration. However, the ultrastructure of the remaining synapses was well preserved, displaying a normal supplement of synaptic vesicles. Therefore, a role of otoferlin in a late step of exocytosis (priming and/or fusion) was postulated (73). A role of otoferlin as Ca^{2+} sensor of vesicle fusion was further suggested by the Ca^{2+} and phospholipid binding of some C_2 domains (73,80), interaction with neuronal SNARE proteins (73,80,81) and facilitation of fusion of SNARE-tagged liposomes (80). These properties are shared by the neuronal Ca^{2+} sensor of fusion synaptotagmin 1, which is lacking from mature inner hair cells (82,83). However, synaptotagmin 1 if introduced as a transgene cannot replace otoferlin in hair cell exocytosis (83). Otoferlin's role in Ca^{2+} regulated fusion should be further addressed by site-directed mutagenesis of the putative Ca^{2+} binding sites (84).

Mutations that do not fully inactivate function have helped further studies of the physiologic role of otoferlin and otoferlin-related synaptopathy. Three mutations were associated with a temperature-sensitive hearing impairment (9,14,76). The affected individuals become deaf when core temperature rises. ABRs at this time are absent. When afebrile, these subjects have a mild elevation of threshold. Speech perception in quiet is normal but impaired in noise. The mechanism underlying the temperature effect still awaits clarification. It might involve protein instability and subsequent degradation possibly leading to a shortage of functional otoferlin. Such a reduction of otoferlin levels (Fig. 4C) was considered as a candidate mechanism for a missense mutation in the region coding the C_2F domain in the *Pachanga* mouse (85). Ca^{2+} dependent vesicle fusion was surprisingly found intact, but a reduced rate of vesicle replenishment was observed (79). In physiology, synapses of inner hair cells replenish hundreds of vesicles per second to enable high rates of transmission and auditory nerve fiber spiking over prolonged periods of time. Interestingly, an additional reduction of vesicle replenishment was found with mice carrying only 1 mutant allele (Fig. 4D, E), in which otoferlin levels were reduced even further. Deafness of these mice was proposed to result from the lack of a sufficient pool of readily releasable vesicles in vivo, when spontaneous release steadily consumes vesicles in excess of the reduced capacity for vesicle replenishment (Fig. 4G). Hence, auditory nerve fibers of these mice could barely respond to sound stimulation (Fig. 4F). In conclusion, aside from being a candidate Ca^{2+} sensor of fusion in hair cells, otoferlin has a function in vesicle replenishment (Fig. 4G). Additional general cell biological functions have been proposed based on protein interaction studies and the broad distribution of otoferlin in hair cells also outside the presynaptic active zones (86–88).

Noise-Induced and Age-Related Hearing Loss

Recent findings indicate that cochlear synaptic mechanisms may contribute to these 2 most common forms of hearing impairment. Changes in synapse number and structure have been implied in noise-induced (89–91) and age-dependent hearing loss (92). Interestingly, a human association study suggests polymorphisms in the gene coding for the metabotropic glutamate receptor mGluR7 to contribute to susceptibility for age-dependent hearing loss (93). Excitotoxic synaptic and neural damage is a key candidate mechanism for noise-induced and age-dependent hearing loss (Fig. 5A). It may result from excessive presynaptic release of glutamate, which has long been discussed for noise-induced hearing loss (see below) and has recently been implied for a human progressive hearing loss caused by mutations in the gene *GIPC3* (94,95). Susceptibility to excitotoxic damage could also arise from abnormally high numbers or sensitivity of postsynaptic glutamate receptors (96), alterations of efferent innervation (97) and from interference with glutamate uptake (98,99), but the relevance of these mechanisms for human disease has not yet been demonstrated.

Excitotoxic synaptic damage due to excessive presynaptic release of glutamate has long been indicated to contribute to noise-induced hearing loss (89–91). Immunohistochemical quantification of ribbon synapse number (28,30) has now been used to establish the loss of ribbon synapses during noise exposures (100,101). Strikingly, even noise exposures that caused only temporary threshold loss were accompanied by a permanent loss of approximately 50% of the hair cell synapses and subsequent slow degeneration of spiral ganglion neurons in the high frequency region of the cochlea (Fig. 5C, D, F, G). The morphologic damage was reflected by a reduced spiral ganglion compound action potential. Measured as Jewett wave I of the auditory brainstem responses, a permanent reduction was found (Fig. 5E), despite full recovery of the physiologic threshold (Fig. 5B). One possible

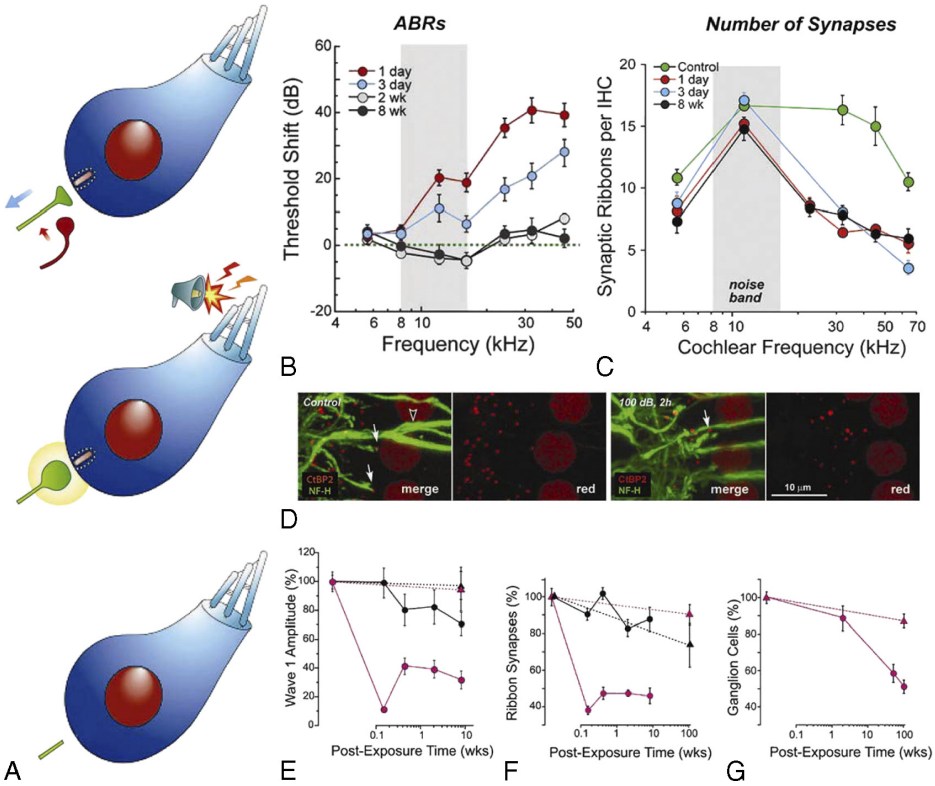

FIG. 5. Excitotoxic irreversible loss of IHC ribbon synapses during noise-induced temporary threshold loss. *A*, Cartoon illustrating excitotoxic synaptic insult: loud noise induces excessive presynaptic glutamate release that causes overexcitation and massive sodium influx into the postsynaptic terminal of the SGN. The ensuing osmotic load causes swelling and finally disruption of the terminal. Work by Kujawa and Liberman (2009) in animals suggests that the SGN do not re-establish synaptic connections with IHCs after the insult and are finally lost. *B*, Induction and recovery of ABR threshold loss following a 100 dB octave band noise for 2 h. *C*, Irreversible loss of half of the synaptic ribbons in high-frequency IHCs in the same mice, despite threshold recovery after 2 weeks. *D*, Representative projections of confocal images of the immunolabeled IHC ribbons in control and noise-exposed mice: reduction of ribbon number. Long-term percentage reduction of (*E*) the amplitude of ABR wave 1 reflecting the loss of synchronously firing SGN, (*F*) ribbon synapse number in high frequency IHCs and (*G*) SGN somata: simultaneous loss of synapses and synchronously firing neurons, delayed physical loss of SGN. (*B*–*G*) were taken with permission from Kujawa and Liberman, *J Neurosci* 2009.

hypothesis explaining this discrepancy of functional findings is that the noise-induced insult hits the low-sensitivity spiral ganglion neurons, which signal loud sounds, but spares the high-sensitivity neurons, which are responsible for sound perception near threshold. This hypothesis can well explain the finding of poor speech recognition in noisy background. Not surprisingly synaptic insult occurs also during noise exposures that cause a permanent threshold increase (100).

Current research aims to understand the presynaptic and postsynaptic changes that occur during noise damage. Moreover, studies explore the reasons why excitotoxic synapse loss is not followed by de novo synapse formation during the weeks after the insult when the disconnected inner hair cells and spiral ganglion neurons are still present. The extent, irreversibility, and functional consequences of excitotoxic synapse loss had not yet appreciated and now require studies of the relevance of this disease mechanism for human noise-induced hearing loss. If comparable to the animal findings, which is likely the case, noise exposure is much more dangerous than we have assumed. We will then have to acknowledge that noise induces synapse and progressive neuron loss and thereby impairs speech reception in noisy environments. We will need to revise noise exposure guidelines, diagnostic procedures and clinical evaluation of occupational hearing loss. In summary, excitotoxic synaptic damage is likely a disease mechanism of noise-induced and possibly also of age-dependent hearing loss (101,102).

DISCUSSION

Identification and Characterization of Auditory Synaptopathy

Auditory synaptopathy-impaired synaptic sound encoding has only recently been appreciated as a disease mechanism of both genetic and acquired hearing impairments. The similarity of clinical expression to auditory neuropathy (15,16): preserved otoacoustic emissions and/or cochlear microphonic potentials reflecting cochlear outer hair cell function but absent or abnormal auditory brainstem responses due

to impaired sound coding led to the initial denomination as auditory neuropathy or auditory neuropathy spectrum disorder. This review describes specific disease mechanisms, focusing on presynaptic alterations at the inner hair cell synapse. Human genetics has uncovered that monogenic defects and complex genetic diseases also affect sound encoding at the hair cell synapses. Starting with the identification of otoferlin (10), an increasing number of defects in genes that code for synaptic proteins and ion channels have been identified, and the list is expected to still increase. Molecular physiology in genetically manipulated mice has provided insights into gene function at the synapse and the synaptic mechanisms underlying the human disease. These studies unambiguously demonstrate the synapse as a primary site of lesion and hence support the use of auditory synaptopathy as the precise nosologic category. However, severe auditory synaptopathy sooner or later leads to degeneration of the spiral ganglion neurons and, thus, has a common final path with primary neural disorders such as hereditary motor and sensory neuropathy.

Understanding Mechanisms and Phenotypes of Auditory Synaptopathies Based on Detailed Analysis of Mouse Models

Mouse models serve as powerful tools for dissecting the precise disease mechanisms, for predicting onset and progression of degeneration and for devising therapeutic approaches. Different from the described mouse models of human auditory synaptopathy, other ''synaptic'' mouse mutants allow one to study the consequences of more subtle synaptic deficits for auditory systems function. Genetic disruption of the presynaptic protein Bassoon causes a mild synaptic hearing impairment (30,103) because of a reduction in the number of releasable synaptic vesicles and Ca^{2+} channels (33). ABR are present but display a massive reduction in wave 1 amplitude (30,103) because of reduced auditory nerve fiber spiking rates and increased jitter of first spike latency (103). Although no human mutations have been described so far, this mouse line has gained considerable interest as a model for auditory synaptopathy.

Otoferlin: Synergistic Research on Human Subjects and Animal Models Advance Our Understanding of Otoferlin Function and Dysfunction

The genetics, structure, and function of otoferlin in the context of hearing and deafness define a hot topic of auditory research. After identifying *OTOF* about a decade ago as the gene defect underlying autosomal recessive, nonsyndromic profound deafness DFNB9 (10), work now encompasses molecular, cellular, and systems level approaches. The presence of human subjects with temperature-sensitive *OTOF* mutations enables advanced electrophysiologic and psychophysical studies and promises to contribute to our understanding of otoferlin-related hearing impairment and auditory synaptopathy in general. Genetic manipulations in mice combined with comprehensive structural and functional analysis will continue to contribute. In particular, these studies will help to further test the Ca^{2+} sensor of vesicle fusion and vesicle replenishment hypotheses.

Presynaptic and Postsynaptic Mechanisms of Synaptopathy

Here, we have reviewed exemplary presynaptic and postsynaptic mechanisms of synaptic hearing impairment with much emphasis on the presynaptic dysfunction. Future research will reveal further genetic and acquired synaptopathies, which will likely also include other alterations of postsynaptic function. Combining specific clinical and genetic testing will likely help to distinguish primarily presynaptic and postsynaptic dysfunctions.

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