Cochlear synaptopathy

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Abstract
Our understanding of cell structure damage in the peripheral auditory system due to acoustic overexposure and ageing underwent a paradigm shift with the discovery, over a decade ago, of cochlear synaptopathy (CS) – the permanent loss of synaptic connections between inner hair cells and auditory nerve fibres. Until then it was upheld that hair cells, and outer hair cells in particular, were the most vulnerable element in the peripheral auditory system. The classical paradigm of clinical audiological assessment has always been - and still is - based on measuring hearing thresholds with pure-tone audiometry. However, the discovery of CS has made it more urgent to develop new and more accurate diagnostic methods to detect hearing damage that is hidden in audiometry and to develop more specific tests for different types of peripheral cell damage. This article reviews the scientific literature on CS in animal models and discusses the evidence of CS in humans from cadaveric studies. Finally, after giving an overview of various inconclusive studies using psychoacoustic and physiological techniques in living humans, the article outlines some of the work currently underway in some European universities and future prospects for diagnosing and treating peripheral hearing loss.

Keywords
Hidden hearing loss, Audiology, auditory nerve, audiogram, synapse, evoked potentials, review article, computational models, Physiology

Clinical implications
Cochlear synaptopathy – the permanent loss of synaptic connections between inner hair cells and auditory nerve fibres – was demonstrated more than 10 years ago in animal models and, more recently, in human cadavers. Synaptic loss is undetectable in pure-tone audiometry, and yet it is highly likely to hinder acoustic signal perception in noisy environments. It is essential for audiology clinicians to be aware of the existence of cochlear synaptopathy and take patients seriously who, despite having normal audiométric thresholds, complain of hearing problems, with phrases like ‘I can hear but not understand’.

How to cite

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Introduction

Pure-tone audiometry has been the gold standard audiological assessment for over seven decades, and its practical utility is universally acknowledged. However, we have known for years that the test is not sensitive to all peripheral auditory system conditions. Elevated hearing thresholds measured by tone audiometry is closely associated with outer hair cell (OHC) loss (Ryan and Dallos, 1975) and may also be associated with inner hair cell (IHC) dysfunction, but not IHC loss (Liberman and Kiang, 1984). In fact, audiometry is extremely insensitive to massive scattered IHC loss (Lobarinas et al., 2013) and also to auditory nerve (AN) fibre loss (Schuknecht and Woeller, 1955). In noise-induced hearing loss it was traditionally upheld that hair cells, and OHCs in particular, were the most vulnerable elements in the peripheral auditory system. Thus, hearing thresholds in the normal range (hearing level (HL) <20 dB at standard frequencies from 125 Hz to 8000 Hz) were understood to indicate a healthy auditory system, because OHCs (the most vulnerable elements) had to be healthy and functional to maintain those thresholds. However, in clinical practice, about 5% of patients complain of difficulties understanding speech, particularly in noisy environments, despite having normal hearing thresholds (<20 dB HL: Hind et al., 2011; Tremblay et al., 2015; Kumar et al., 2007; Saunders and Haggard, 1989; Cantuaria et al., 2021). This clinical evidence suggests the presence of some type of auditory pathway dysfunction, which often used to be attributed to central (i.e., brainstem) or cortical neural structures, and not to the periphery. This condition was given the name of hidden hearing loss (Schaette and McAlpine, 2011).

The aim of this paper is to review the most relevant scientific literature on hidden hearing loss and, more specifically, on cochlear synaptopathy (CS; see definition below). The review draws on the author's research experience in this field since 2014, but spans from the discovery of CS in 2009 in the mouse model (Kujawa and Liberman, 2009) to the present day. The paper focuses on the definition of CS based on animal model studies, on the development of diagnostic techniques, and on work undertaken to demonstrate and measure the presence of CS in living humans. Molecular aspects and structural changes in CS, and the development of imaging techniques are beyond the scope of this review. A longer version of this review can be found as a chapter in Manual de audiología laboral [Manual of occupational audiology] by Peñuela et al. (2022). A review of more recent, complementary literature in the field can be found in an article by Liu et al. (2024). The present review is structured as follows: the first part focuses on studies of noise-exposure CS in animal models, and the effects of aging on synapse loss in the AN, also in animal models. It continues with an overview of the different studies on hidden hearing loss in humans, noting the variability and divergence of results, which are inconclusive. The last part looks into the future and at prospective diagnostic and pharmacological techniques for managing CS in humans.

Noise-induced cochlear synaptopathy

Cochlear synaptopathy is described as a permanent disconnection, interruption or loss of the synapses connecting with the IHCs in the cochlea (Kujawa and Liberman, 2009). The first report of CS was from a study on noise-exposed mice subjected to 105 dB sound pressure level (SPL) for two hours, producing an immediate temporary elevation of hearing thresholds of about 30-40 dB (measured by distortion product otoacoustic testing [DPOAE] and auditory brainstem responses [ABR]), which returned to pre-exposure values about two weeks post-exposure (Figure 1H and I). Using imaging techniques, the authors viewed different cochlear regions and counted the IHCs, AN fibres and synaptic receptors paired to synaptic ribbons in the IHCs (Figure 1A and B). Each peripheral axon of the AN (green filaments, Figure 1A and B) makes a single synaptic connection with a synaptic ribbon (red dots, Figure 1A and B) in one IHC (IHC nucleus in blue, Figure 1A and B). Synapse losses of 50-55% were found in the basal region of noise-exposed mice compared with the control mice. While the DPOAE and ABR-wave-I measured thresholds returned to pre-exposure values (Figure 1H and I), synaptic losses were permanent (i.e., no synapse recovery was found over time). In addition, no evidence of IHC or OHC loss was found in any cochlear region. In this respect, no differences were found between exposed and control animals in DPOAE amplitude measurements by stimulation level (Figure 1H), since the OHCs were intact in the...
exposed animals. However, noise-exposed animals did show permanent functional effects in the form of a permanent reduction of the supra-threshold levels of ABR wave I (Figure 1). Later studies have demonstrated a high correlation between ABR wave I amplitude and cochlear synapse survival (Sergeenko et al., 2013; Parthasarathy and Kujawa, 2018). The presence of CS has been demonstrated in other mammals such as guinea pigs (Lin et al., 2011; Liu et al., 2012), rats (Lobarinas et al., 2017), chinchillas (Hickman et al., 2018; Hickox et al., 2017), Rhesus macaques (Valero et al., 2017) and humans (Makary et al., 2011; Viana et al., 2015; Wu et al., 2019).

After the initial discovery of CS, subsequent studies found that not all AN fibres were equally affected. A study by Furman et al. (2013) suggested that synapse loss was much more selective for fibres with low- and medium-spontaneous rates (SR), hardly affecting those with high-SR. In the AN, two to three subgroups of afferent neurons can be identified according to their spontaneous discharge rate (i.e., the number of action potentials generated in the absence of stimulation). In cats, which are sensitive to low and medium frequency ranges, three types of neurons have been demonstrated: high-SR neurons (more than 18 discharges/second), medium-SR neurons (between 0.5 and 18 discharges/second) and low-SR neurons (less than 0.5 discharges/second; Liberman, 1978). In other mammals sensitive to higher frequency ranges, such as mice, two types of neurons have been described: high-SR (>1 discharge/second) and low-SR (<1 discharge/second); Taberner and Liberman, 2005). It is thought that the characteristics of the human AN resembles the cat AN more than the mouse AN, but this is still to be demonstrated. Neuronal spontaneous discharge rate is associated with the neuronal excitation threshold. High-SR neurons are sensitive to low thresholds, while low-SR neurons are sensitive to high thresholds (Liberman, 1978). In addition to these functional differences, there are also morphological differences. The same IHC receives synaptic connections from all three types of AN neurons (high-, medium- and low-SR neurons). However, low-SR neurons tend to innervate the modiolar side of the IHC, whereas high-SR neurons tend to innervate the pillar side. In addition, low-SR fibres tend to have thinner axons and fewer mitochondria, whereas high-SR fibres have thicker axons and more mitochondria (Liberman, 1982). The study by Furman et al. (2013) used these modiolar/pillar gradients in AN synapse innervation (i.e., the IHC side on which fibres are innervated) in exposed animals versus controls. They concluded that there was more loss of low- and medium-SR fibres in exposed animals. This finding was also corroborated by direct measurements of individual fibres that showed differences in the statistical distributions of spontaneous discharge rate between the control and exposed animals.

The study by Furman et al. (2013), which found that CS was selective for low- and medium-SR fibres, had a major impact on the design of both electrophysiological and psychoacoustic experiments in humans. This also explained why hearing thresholds were preserved in CS, and why, on the contrary, supra-threshold responses (i.e. high-level ABR wave I amplitude) were indeed affected. If CS did not affect high-SR and low threshold fibres, the fibres could still encode the low intensity signals used in threshold measurements; at the same time, the selective loss of low-SR and high-threshold neurons would lead to problems encoding the acoustic signal at supra-threshold levels. However, some authors began to question these results and proposed that CS affected all AN fibres, regardless of spontaneous discharge rates. Indeed, a reanalysis of the data by Furman et al. (2013) found that in the same original study there was actually a loss of high-SR fibres of more than 26%, which had not been clearly reported (Marmel et al., 2015). In addition, most studies that used computational models to predict the effect of CS have been forced to apply significant loss affecting high-SR fibres (Paul et al., 2017; Verhulst et al., 2018; Encina-Llamas et al., 2019; Keshishzadeh et al., 2020, 2021; Johannesen et al., 2022). A more recent study conducted in mice has provided evidence contrary to what was suggested by Furman et al.(2013), finding that CS was not selective for low- and medium-SR fibres (Suthakar and Liberman, 2021). The study measured the direct response of single fibres in control mice and in noise-exposed mice sustaining more than 50% loss with CS. Measurements in surviving AN fibres in the noise-exposed mice showed no difference in the statistical distributions of spontaneous discharge rate or in neuronal properties versus measurements in the control mice, suggesting that all three fibre types sustained similar losses from sound exposure. It is interesting that this 2021 study was conducted in mice, like the original CS study by Kujawa and Liberman (2009), whereas the study by Furman...
et al. (2013) was done in guinea pigs. Recently, it has been shown that over time, guinea pigs are able to regenerate synapses lost immediately after noise exposure (Hickman et al., 2020, 2021; Shi et al., 2013), which may explain the discrepancy between studies in mice and guinea pigs and computational models. In other species such as chinchillas, no evidence of synaptic regeneration has been observed (Bharadwaj et al., 2022). In humans, studies in temporal bones obtained at autopsy show clear synaptic loss with age, with a fibre degeneration rate similar to that in mice (Wu et al., 2019, 2020), suggesting an absence of cochlear synaptic regeneration in humans.

Age-related cochlear synaptopathy

As part of the ageing process, the number of active synaptic connections between the AN and the IHCs declines naturally. In healthy ageing animals, synaptic loss has been shown to occur steadily and continuously over the animal’s lifespan, reaching a 50% loss in the oldest specimens (Sergeyenko et al., 2013). As in the case of noise-induced CS, age-related CS precedes hair cell loss and threshold elevation (Figure 1F), which are minimal until advanced age. Synaptic loss is followed by the corresponding spiral ganglion cell loss, but with a time lag (Figure 1F) that is very similar to the degeneration of the entire AN fibre also found in human temporal bones (Makary et al., 2011). Noise exposure accelerates this natural age-related synaptic loss (Fernandez et al., 2015). The functional effects of age-related CS are similar to those of noise-induced CS: DPOAEs remain unchanged providing there is no OHC loss, the ABR wave I amplitude is reduced as the individual ages, in clear correlation with the synapse count (Sergeyenko et al., 2013), and the amplitude of steady-state evoked potentials such as envelope-following responses (EFR) decreases with age in correlation with the synapse count (Parthasarathy and Kujawa, 2018).

Cochlear synaptopathy in humans

The existence of cochlear synaptopathy in humans has been the subject of much debate among the scientific community in the past (Bramhall et al., 2019), and was finally demonstrated in human histopathological studies on temporal bones obtained at autopsy (Wu et al., 2019, 2021). These studies showed a clear age-dependent loss of synapses in humans, similar to that in animals, and occurring at a higher rate than in prior hair cell loss. However, attempts to find evidence of CS in living humans have been much more controversial (Valderrama et al., 2022), because studies in living humans have several additional difficulties compared to studies in animals: 1) human genetics shows much greater diversity than some rodents, especially mice, which are almost genetic copies of each other. Genetic diversity causes greater variability in the individual effects of an auditory system insult, such as noise exposure, and greater variability in potential biomarkers; 2) in animal studies, researchers have worked long and hard to find a noise intensity and exposure time that causes significant synapse loss without hair cell loss. It is therefore possible to study CS in laboratory animals in complete isolation from other pathologies, whereas in humans a controlled environment is almost impossible, and various pathologies (e.g., CS, OHC and IHC loss and/or dysfunction, stria vascularis degeneration and spiral ganglion cell loss) are inevitably present concomitantly in the same individual; 3) in living humans it is ethically impossible to perform a histopathological study to count cochlear synapses. In short, real evidence is impossible in living humans.

Researchers have tackled these problems using four main strategies:

A) They have assessed lifetime noise exposure through questionnaires, relating it to one or more physiological biomarkers sensitive to CS in animals. Most of these studies used ABR wave I amplitude as a biomarker of CS and did not find clear and significant correlations between wave I amplitude and sound exposure estimation (Prendergast et al., 2017a; Stamper and Johnson, 2015; Fulbright et al., 2017; Spankovich et al., 2017; Grinn et al., 2017; Ridley et al., 2018; Maele et al., 2021). Other studies have used dosimeters to measure sound exposure, with the disadvantage that sound exposure estimation is obviously limited by time. These studies either found no effect or only small effects on ABR latency (Skoe and Tufts, 2018; Maele et al., 2021). Other studies used EFR magnitude as a biomarker without finding any association (Prendergast et al., 2017a; Guest et al., 2017b, a; Grose et al., 2017). However, some studies have found some association between noise
exposure and a CS-related physiological response, but mostly showed weak effects. For example, effects on wave I amplitude have been reported (Valderrama et al., 2018; Bramhall et al., 2018a) on the relationship between the summing potential (SP) and the action potential (AP; equivalent to the ABR wave I) (Liberman et al., 2016; Grose et al., 2017), and on EFR magnitude (Bharadwaj et al., 2015; Bramhall et al., 2021).

B) The second strategy was also based on assessing patients’ lifetime noise exposure through questionnaires and relating it to one or more CS-associated speech perception measures by means of heuristic argumentation, i.e., following an undemonstrated logical composition of arguments, such as that CS degrades speech intelligibility in noise (Lopez-Poveda and Barrios, 2013; Lopez-Poveda, 2014). Most of these studies have shown no clear, significant correlation between sound exposure estimation and various behavioural hearing tests presumed to be affected by cochlear synapse loss (Prendergast et al., 2017b; Yeend et al., 2017; Prell et al., 2018; Fulbright et al., 2017; Grinn et al., 2017; Mael et al., 2021; Grose et al., 2017; Guest et al., 2018). One study did show worse speech-in-noise intelligibility among young music students assigned to the high-risk group based on self-report for noise exposure (Liberman et al., 2016).

C) The aim of the third strategy was to find correlations between various physiological and behav-
jural measurements thought to be sensitive to CS, derived from animal studies. Again, a number of studies showed no significant correlations between wave I amplitude and speech-in-noise intelligibility (Fulbright et al., 2017; Grinn et al., 2017; Maele et al., 2021; Prendergast et al., 2017b; Guest et al., 2018; Bramhall et al., 2018a; Johannesen et al., 2019), EFR magnitude (Maele et al., 2021; Prendergast et al., 2017b; Guest et al., 2018) or the relationship between ABR I- and V-wave amplitudes and speech-in-noise intelligibility (Guest et al., 2018). However, one study did report significant correlation with speech-in-noise intelligibility. For example, an association was found between SP and AP (or wave I amplitude) and speech-in-noise intelligibility (Liberman et al., 2016; Grant et al., 2020), although the reliability of this measure has been found to lack robustness (Prendergast et al., 2018). Speech intelligibility has also been associated with EFR magnitude (Mepani et al., 2021). Another study showed worsening of speech intelligibility in subjects who had longer interpeak latencies for ABR waves I to V (an indicator of neural transmission time between the AN and inferior colliculus). The same subjects also had lower wave I to V amplitude ratios (indicative of elevated central gain, see below); Valderama et al., 2018). Finally, a correlation was reported between the wave-V latency of ABRs measured in masking noise and the detection of interaural timing difference in the envelope of the acoustic stimuli (Mehraei et al., 2016).

D) The fourth and last strategy was to find a relationship between the existence of CS, based on measurements derived from animal studies, and the presence of tinnitus. Different studies have shown that, despite a reduction in ABR wave I amplitudes in normal ears presumably related to the existence of CS, wave-V amplitude remain unchanged (Burrard and Sims, 2001; Johannesen et al., 2019; Grose et al., 2019; Rumschlag et al., 2022; Johannesen and Lopez-Poveda, 2021; Schaette and McAlpine, 2011; Temboury-Gutierrez et al., 2024b). This phenomenon has been related to the concept of central gain, which would explain overexcitation of the central auditory system (i.e., the brainstem) as a result of reduced central neuronal inhibition (Heeringa and van Dijk, 2014) compensating the reduced peripheral activity due to synapse loss (Chambers et al., 2016; Auerbach et al., 2014; Sheppard et al., 2018; Mohrle et al., 2019; Johannesen and Lopez-Poveda, 2021; Salvi et al., 2017; Caspary et al., 2008; Lai et al., 2017; Parthasarathy et al., 2019; Diehl and Schaette, 2015), which would also cause heightened cortical activity (Zan et al., 2020). One current hypothesis links central overexcitation to the presence of tinnitus; i.e, the perception of sound in the absence of a real external sound source (Mohrle et al., 2016; Eggermont, 2017; Knipper et al., 2013; Schaette, 2014; Schaette and McAlpine, 2011). Some CS biomarkers have also been associated with tinnitus, such as the acoustic reflex (Wojtczak et al., 2017), but a later study found no such link (Guest et al., 2019). Reduced EFR magnitude has also been associated with tinnitus (Paul et al., 2017), but a review of the same study ultimately found that the effect was not statistically significant (Roberts et al., 2018).

In summary, CS studies in living humans give contradictory and therefore inconclusive results. This status quo calls for new, more imaginative studies combining different techniques and tests, making use of the latest technology and computing power.

**Prospective treatments and diagnostic techniques**

Today’s treatments are unable to completely reverse hearing loss, although solutions exist to compensate or alleviate the effects of hearing loss through auditory rehabilitation using hearing aids or cochlear implants. However, there is speculation that in the coming decades, pharmacological solutions may reverse or prevent some hearing impairments. These speculations need three things to happen at the same time to become a reality: 1) specific and efficient drugs must be developed to restore damaged cells safely and without side effects, backed by the corresponding clinical trials; 2) surgical techniques need to be developed to deliver drugs in an efficient and controlled way to the damaged cochlear sections; and 3) accurate diagnostic techniques need to be developed to assess the degree of degeneration and damage to the different cell types in the peripheral auditory system in individual patients. In recent years, various research projects on CS have focused on the use of neurotrophic factors in neuronal regeneration (Cassinotti et al., 2022; Suzuki et al., 2016; Foster et al., 2022b; Leake et al., 2020; Hashimoto et al., 2019), some of which have reached different stages in clinical trials (Foster et al., 2022a). The aim of these techniques is to regenerate synapses on AN fibres that have been disconnected, and achieve reconnection...
to the corresponding IHC. Some authors, albeit fewer, have reported on transtympanic techniques and strategies to deliver drugs locally to the cochlea (Maxwell et al., 2021; Foster et al., 2022a). In addition, some surgeons at Rigshospitalet hospital in Copenhagen, Denmark, and others elsewhere, are developing intra-cochlear drug delivery surgical techniques in conjunction with other researchers in the Danish audiology sector (author’s private sources).

Some researchers are making remarkable progress in individualised, targeted diagnostics, even though no techniques currently exist to clinically assess CS. As mentioned earlier, CS in humans is most likely to occur together with other types of cochlear cell loss or dysfunction. Solutions are therefore likely to need a combination of different experimental measurements, probably assisted by computational modelling, together with efficient use of artificial intelligence (AI) models. Along these lines, various studies have used computational models to predict the effect of CS and other hearing losses on different physiological responses (Paul et al., 2017; Keshishzadeh et al., 2020; Verhulst et al., 2018; Encina-Llamas et al., 2019, 2021; Märcher-Rørsted et al., 2022). Recently, researchers conducting studies at Ghent University in Belgium have developed a framework that combines computational physiological models with AI models to develop new auditory processing strategies to compensate some auditory pathologies, such as CS and OHC loss (Bramhall et al., 2018b; Buran et al., 2022; Drakopoulos et al., 2021, 2022; Drakopoulos and Verhulst, 2023; Drakopoulos et al., 2023). Briefly, good computational physiological models exist that predict the AN response to any acoustic stimulus (Bruce et al., 2018; Verhulst et al., 2018) and AI models (neural networks) can be trained to produce responses that are almost identical to the physiological model. These researchers built two AI models: a healthy model to simulate a normal-hearing AN response and a hearing-impaired one with a hearing impairment simulating a damaged response. Equipped with these two responses, they are then able to build a third AI model coupled to the input of the hearing-impaired model with the aim of reducing the difference between the hearing-impaired response and the normal-hearing response (see Figure 1 in Drakopoulos and Verhulst, 2023), so that the hearing-impaired model response resembles the normal-hearing model response (to compensate the loss). The result lends itself to ideal hearing aid processing. The authors noted that OHC losses are more easily compensated than synapse losses.

Concurrently, research groups that the present author is affiliated with at the Technical University of Denmark (DTU), together with the Copenhagen Hearing and Balance Center (CHBC) at Rigshospitalet hospital in Copenhagen, have been refining the framework model developed by Dau (2003) to simulate cochlear electrophysiological responses (through electrocochleography, ECochG) in both healthy and various peripheral hearing-loss profiles (Temboury-Gutierrez et al., 2024a). The aim of this research is to develop an AI model based on one or more ECochG responses in an individual patient that can predict which combination of CS, IHC dysfunction and OHC loss/dysfunction is most likely to be present. To do this, it is essential to develop computational models that accurately predict the AN response in humans. Based on these investigations, evoked potentials such as frequency-following responses (FFR) may potentially be a sensitive biomarker of CS and also robust to other losses, such as OHC loss or dysfunction, as corroborated in computational models (Märcher-Rørsted et al., 2022; Temboury-Gutierrez et al., 2024b). Similar studies in the chinchilla animal model appear to support these results in humans. An alternative type of evoked potential are EFRs (Encina-Llamas et al., 2019; Keshishzadeh et al., 2020, 2021; Vasilkov et al., 2021), which were shown to be sensitive to CS in mice (Parthasarathy and Kujawa, 2018). In short, it appears that a combination of steady-state evoked potentials with different types of advanced computational models may be the key to making accurate hearing diagnoses in humans. When these new methods have been demonstrated and replicated, we will then need to find ways to adapt them to the needs of a clinical practice setting.

**Conclusion**

The classical paradigm regarding damage from acoustic overexposure and ageing upheld that OHCs were the most vulnerable element in the peripheral auditory system. In 2009, it was demonstrated in mice models that CS – the permanent loss of synaptic connections between IHCs and AN fibres – preceded hair cell loss. This synaptic loss does not affect hearing thresholds and is therefore hidden on pure-tone audiometry. However, it does cause a reduction in
the supra-threshold response in the AN and is likely to hinder sound perception in noisy environments. Cochlear synaptopathy has been demonstrated in several mammals, including humans. It occurs naturally as part of the ageing process and is exacerbated by sound overexposure. Studies in humans using psychoacoustic measurements lack full consensus in their results. More recently, some steady-state evoked potentials such as EFRs and FFRs have shown more potential to be considered as good biomarkers sensitive to CS. It is anticipated that in the next few years, these evoked potentials will be combined with physiological computational models and AI models to help diagnose CS in humans accurately and reliably.

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Conflict of interest
The author declares no conflicts of interest.

Funds
This work has been funded by a grant from the GN Foundation (ref. 237) as part of the AudPhen (Auditory Phenotypes in Denmark) at the Rigshospitalet hospital in Copenhagen (Denmark).