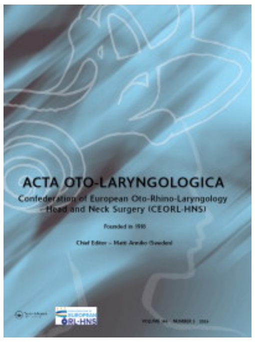

Acta Oto-Laryngologica

ISSN: 0001-6489 (Print) 1651-2251 (Online) Journal homepage: www.tandfonline.com/journals/ioto20

Oxidative stress and inflammation in age-related hearing loss: temporal dynamics in cochlear degeneration of C57BL/6 mice

Anna Pisani, Brenda Hassler, Fabiola Paciello, Veronica Mohamed Hizam, Gaetano Paludetti, Claudio Grassi & Anna Rita Fetoni

To cite this article: Anna Pisani, Brenda Hassler, Fabiola Paciello, Veronica Mohamed Hizam, Gaetano Paludetti, Claudio Grassi & Anna Rita Fetoni (2026) Oxidative stress and inflammation in age-related hearing loss: temporal dynamics in cochlear degeneration of C57BL/6 mice, Acta Oto-Laryngologica, 146:3, 344-350, DOI: 10.1080/00016489.2025.2598401

To link to this article: https://doi.org/10.1080/00016489.2025.2598401

ACTA OTO-LARYNGOLOGICA
2026, VOL. 146, NO. 3, 344–350
https://doi.org/10.1080/00016489.2025.2598401

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Oxidative stress and inflammation in age-related hearing loss: temporal dynamics in cochlear degeneration of C57BL/6 mice

Anna Pisani[a#], Brenda Hassler[a#], Fabiola Paciello[b,c], Veronica Mohamed Hizam[d], Gaetano Paludetti[c,d], Claudio Grassi[b,c] and Anna Rita Fetoni[a]

[a]Department of Neuroscience, Unit of Audiology, Università degli Studi di Napoli Federico II, Naples, Italy; [b]Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy; [c]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [d]Department of Head and Neck Surgery, Università Cattolica del Sacro Cuore, Rome, Italy

ABSTRACT

Background: Age-related hearing loss (ARHL) is a condition linked to cochlear degeneration influenced by genetic, environmental, and comorbid factors. Although oxidative stress and inflammation have been implicated in ARHL, their dynamic interplay during aging remains unclear.
Aims/Objectives: This study aimed to clarify the temporal progression of oxidative and inflammatory mechanisms in cochlear degeneration using the C57BL/6 mouse model of ARHL.
Material and Methods: C57BL/6 mice, characterized by a predictable auditory decline, were examined at 3, 6, and 12 months of age. Auditory brainstem responses (ABRs) were recorded to assess hearing thresholds and neural transmission efficiency. Morphological analyses were performed to evaluate neuronal integrity, while cochlear tissues were analyzed for markers of oxidative stress and inflammation, including 3-nitrotyrosine (3-NT), 4-hydroxynonenal (4-HNE), and tumor necrosis factor-alpha (TNF-α).
Results: With advancing age, mice exhibited a progressive elevation of hearing thresholds, reduced neural transmission efficiency, and loss of spiral ganglion neurons. Molecular analyses revealed increased 3-NT, 4-HNE, and TNF-α expression. Both oxidative stress and inflammation were detectable from 6 months of age, suggesting that these processes synergistically drive cochlear degeneration.
Conclusions and Significance: Our findings underscore the role of oxidative stress-inflammation crosstalk in ARHL, suggesting therapeutic targets for preventing presbycusis in aging populations.

背景

背景:　年龄相关性听力损失(ARHL)是一种与耳蜗退行性病变相关的疾病,受遗传、环境和合并症因素的影响。尽管氧化应激和炎症与ARHL有关,但它们在衰老过程中的动态的相互作用仍不清楚。
目的:本研究旨在利用C57BL/6 ARHL小鼠模型,弄清耳蜗退行性病变中氧化和炎症机制的时间进程。
材料与方法:　本研究选取具有可预测听力下降特征的C57BL/6小鼠,分别在3、6和12月龄时进行检查。记录听觉脑干反应(ABR)以评估听阈和神经传递效率。形态学分析用于评估神经元完整性,同时分析耳蜗组织中氧化应激和炎症标志物,包括3-硝基酪氨酸(3-NT)、4-羟基壬烯醛(4-HNE)和肿瘤坏死因子-α(TNF-α)。
结果:　随着年龄增长,小鼠的听阈逐渐升高,神经传递效率降低,螺旋神经节神经元消失。分子分析显示3-NT、4-HNE和TNF-α的表达增加。氧化应激和炎症在6月龄时即可检测到,表明这些过程协同驱动耳蜗退化。
结论和意义:　我们的研究结果强调了氧化应激-炎症相互作用在年龄相关性听力损失(ARHL)中所起的作用,提示了预防老年人群老年性耳聋的潜在治疗靶点。

ARTICLE HISTORY
Received 13 October 2025
Revised 25 November 2025
Accepted 26 November 2025

KEYWORDS
Age-related hearing loss; cochlea; oxidative stress; inflammation

Introduction

ARHL is a progressive decline in auditory sensitivity associated with the degeneration of cochlear receptors. Its onset is multifactorial, involving genetic predisposition, environmental risk factors, such as chronic noise exposure and ototoxic drugs, as well as comorbid conditions [1,2]. Clinically, ARHL typically begins in the high-frequency range, which is among

CONTACT Anna Rita Fetoni annarita.fetoni@unina.it Department of Neuroscience, Unit of Audiology, Università degli Studi di Napoli Federico II, Naples, Italy, 80131.
#Authors share the first authorship.

the first to deteriorate with aging, and then extends to lower frequencies. Although widely studied, the molecular mechanisms driving presbycusis remain complex and not fully elucidated.

One major contributor to cochlear aging is the progressive accumulation of oxidative damage caused by reactive oxygen species (ROS) [3]. This process promotes mitochondrial dysfunction, reduced energy production, and tissue degeneration. Under physiological conditions, antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione S-transferase (GST), and glutathione peroxidase (GPX), maintain redox balance [4]. However, with aging, the imbalance between ROS production and antioxidant defenses leads to oxidative stress, which not only exacerbates cellular injury but also triggers inflammatory pathways. ROS activate nuclear factor kappa B (NF-κB) and other redox-sensitive transcription factors, enhancing the expression of pro-inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) [5]. This persistent low-grade inflammation, or 'inflammaging,' contributes to cellular senescence and tissue dysfunction [6]. Nevertheless, the precise timing and causal interplay between oxidative stress and inflammation in the aging cochlea remain unclear.

C57BL/6 mice are a widely used model of ARHL, as they display a predictable progression of hearing loss that starts with high frequencies and gradually extends across the auditory spectrum [7,8]. These mice carry genetic mutations predisposing them to early-onset ARHL, making them particularly suitable for exploring the molecular mechanisms of presbycusis. Their phenotype shares several hallmarks with human ARHL, including synaptic dysfunction, hair cell loss, and impaired neural transmission.

In this study, we employed C57BL/6 mice to investigate the contribution of oxidative stress and inflammation to cochlear degeneration. By examining these processes across different ages, we aimed to define the kinetics of damage and identify a potential 'therapeutic window' for interventions targeting oxidative and inflammatory pathways, to mitigate ARHL and preserve auditory function in aging populations.

Materials and methods

Animals

Male adult C57BL/6 mice (Charles River Laboratories, Lecco, Italy) were used. Only male C57BL/6 mice were used in this study to ensure experimental consistency and to minimize the confounding influence of sex-related hormonal factors on cochlear pathophysiology. A total of 15 animals aged 3, 6, and 12 months (M) were studied. Mice were housed 3–5 per cage with ad libitum access to food (Mucedola 4RF21, Italy) and water, under controlled temperature (22–23 °C), constant humidity (60 ± 5%), and a 12-h light/dark cycle. All procedures were made to minimize animal suffering and to reduce their number, in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). All procedures were performed in compliance with the Laboratory of Animal Care and Use Committee of the Catholic University, School of Medicine of Rome, and were approved by the Italian Department of Health (Ministero della Salute, Prot. 1F295.169.EXT.112).

Auditory brainstem responses (ABRs)

Auditory function was evaluated by ABR recordings at low (6 kHz), mid (12, 16, 20 kHz), and high (24, 32 kHz) frequencies at 3, 6, and 12 M. Mice were anesthetized (ketamine 35 mg/kg, medetomidine-domitor 0.25 mg/kg) and placed in an anechoic room. As described previously [9,10], tone bursts were presented monaurally in an open field *via* a horn tweeter (Tucker-Davis Technologies, TDT). ABRs were recorded using three subcutaneous stainless steel electrodes (active: posterior to the tested pinna; reference: vertex; ground: contralateral pinna). Auditory stimuli were generated and ABRs recorded using a PC-controlled Tucker-Davis Technologies (TDT) System 3 (Alachua, FL, United States) with real-time digital signal processing. Tone bursts of pure tones from 6 to 32 kHz (1 ms rise/fall time, 10 ms total duration, 20/s repetition rate) were presented monaurally. The responses were filtered, digitized, and averaged across 512 discrete samples at each frequency-level combination. Wave I and wave II amplitude–intensity (A–I) and latency–intensity (L–I) functions were analyzed to assess auditory nerve fiber integrity, following Fetoni et al. [11,12].

Morphological analysis: H&E staining

Histological changes in SGNs were assessed on cochlear cryosections (12 μm) obtained as previously described [10]. Cochleae were fixed in 4% paraformaldehyde (PBS, pH 7.5, 4 °C), decalcified for 15 days in 10% EDTA, cryoprotected in 30% sucrose for 48 h, embedded in OCT, and sectioned using a Cryostat CM1950 (SLEE). Sections were stained with hematoxylin and eosin (H&E; Abcam, ab245880) using a standard protocol (4–5 min hematoxylin, 45 s eosin) and mounted with Entellan® (Merck, Cat. No. 107960).

The cross-sectional area of Rosenthal's canal in the middle turn was measured with NIH Image. Neuronal profiles per section area were calculated within a 500 × 500 µm area using NIH ImageJ 1.43 u. Counts were performed by two independent, blinded investigators. Neuronal survival was expressed as a percentage relative to 3 M controls.

Western blot and dot blot analyses

Cochlear tissues from 3 animals/groups were homogenized in lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.4, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, protease inhibitor cocktail). Homogenates were sonicated (10 s on/20 s off, 3 cycles; Diagenode Bioruptor Standard) and centrifuged at 22,000 × g and 4 °C. Equal amounts of protein were boiled and resolved using SDS-PAGE, as previously described [5,10,12]. For dot blotting, 5 µl of lysate (5 µg/µl) was spotted onto TBST-prewetted nitrocellulose membranes [5,10]. Membranes were incubated overnight with primary antibodies against 3-nitrotyrosine (3-NT, 1:1000; Sigma-Aldrich, Cat. No. 05–233), 4-HNE (Alpha Diagnostic International, Cat. No. HNE11-S), and TNF-α (1:1000; Santa Cruz Tech., Cat. No. sc-52746), followed by HRP-conjugated secondary antibodies (1:5000; Cell Signaling Tech., Cat. No. 70765) for 1 h at RT. After TBST washes, signals were visualized using chemiluminescent substrates (Cyanagen, Bologna, Italy) and quantified with UVItec Cambridge Alliance. Equal protein loading was verified using GAPDH (SDS-PAGE) and Ponceau S (dot blot).

Statistical analyses

Sample size was determined by power analysis to achieve 80% power at α = 0.05 based on pilot data and previous work. Data were analyzed blinded using Prism 8.0 (GraphPad Software, San Diego, CA, USA). One- or two-way ANOVA followed by Tukey's *post hoc* test was applied when appropriate. *Post hoc* tests were performed only when ANOVA showed significant effects ($p < 0.05$), and variance homogeneity was verified. Results are presented as mean ± SEM, and $p < 0.05$ was considered statistically significant.

Results

Auditory dysfunction with advancing age

To evaluate the effects of aging on auditory function, ABRs were recorded in C57BL/6 mice at 3, 6, and 12 M (Figure 1). Mean ABR thresholds (Figure 2(A)) showed a progressive hearing loss that initially affected high frequencies at 6 M and extended to all frequencies by 12 M. At 3 M, thresholds ranged between 30 and 40 dB across all frequencies. At 6 M, thresholds increased to ~ 50 dB at low/mid frequencies and ~ 60 dB at high frequencies, consistent with early presbycusis. By 12 M, thresholds exceeded 60 dB across the spectrum, confirming the progressive ARHL phenotype in this strain. To further characterize functional decline, we analyzed ABR waves I and II at 16 kHz. Wave I amplitude, reflecting inner hair cell–auditory nerve synaptic integrity, was significantly reduced in 6- and 12-month-old mice (Figure 2(C)), while latency increased with age (Figure 2(D)), suggesting cochlear synaptopathy and impaired neural conduction. Similarly, wave II amplitude declined significantly (Figure 2(E)), accompanied by increased latency (Figure 2(F)), indicating reduced afferent input and delayed signal processing in the cochlear nucleus.

Together, these changes demonstrate age-related neural dysfunction, characterized by reduced neural synchrony, impaired transmission efficiency, and progressive loss of functional auditory units.

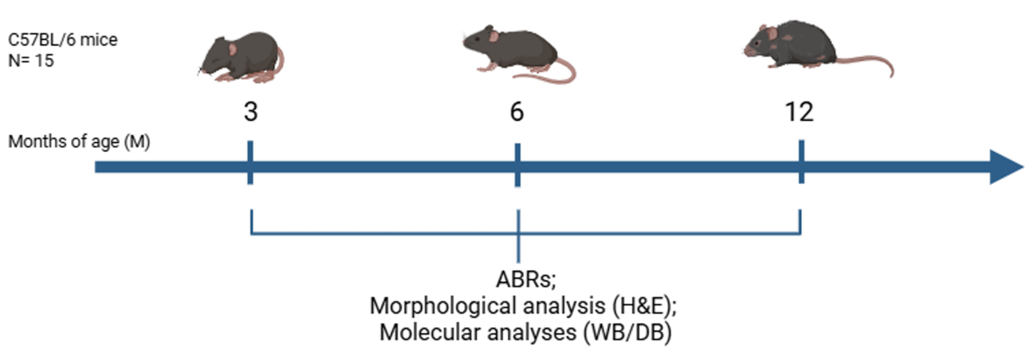

Figure 1. Experimental design and time schedule.
Schematic representation of the experimental design. C57BL/6 mice of 3, 6, and 12 months (M) of age were used in this study. Auditory function was assessed during aging. Molecular analyses (WB/DB) were performed to analyze the modulation of oxidative stress and inflammation during aging. ABR: Auditory Brainstem Responses; H&E: Hematoxylin and Eosin; WB: Western Blot; DB: Dot Blot. Created using Biorender License.

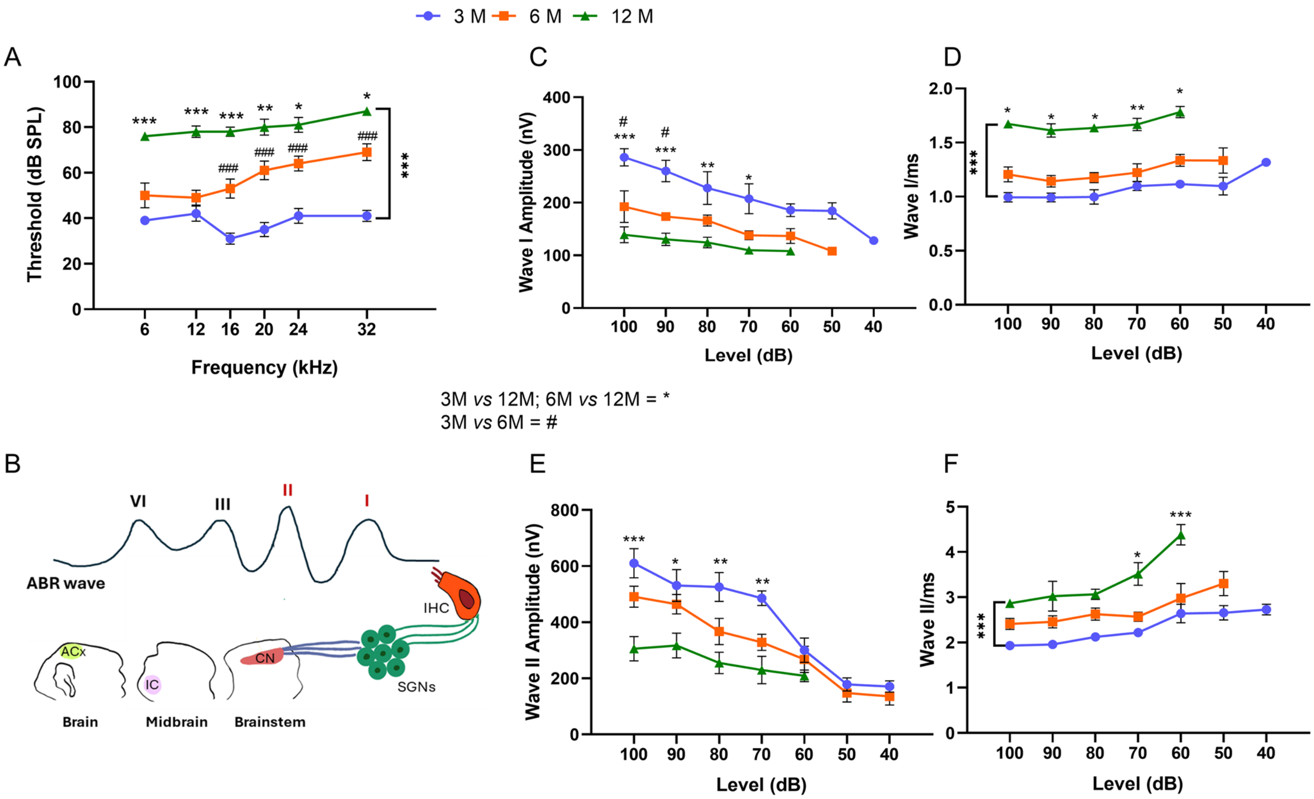

Figure 2. Age-related decline in auditory function in C57BL/6 mice.
A: Graph shows ABR threshold values (means ± SEM) in C57BL/6 mice of 3, 6, and 12 months of age (M). A progressive worsening of auditory threshold was observed in mice during physiological aging. B: Schematic representation of ABR waves. C, E: Amplitude-intensity curves (mean ± SEM) showing a decreased amplitude of wave I (C) and wave II (E) in mice of 6 and 12 M at 16 kHz compared with mice of 3 M. D, F: L–I curve (mean ± SEM) of the P1 (D) and P2 (F) waveform (16 kHz) showing increased latency of waves during physiological aging. Asterisks indicate significant differences among groups (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) from two-way (A) and one-way ANOVA (B,C) with repeated measures. IHC: Inner Hair Cell; SGNs: Spiral Ganglion Neurons; CN: Cochlear Nucleus; IC: Inferior Colliculus; ACx: Auditory Cortex.

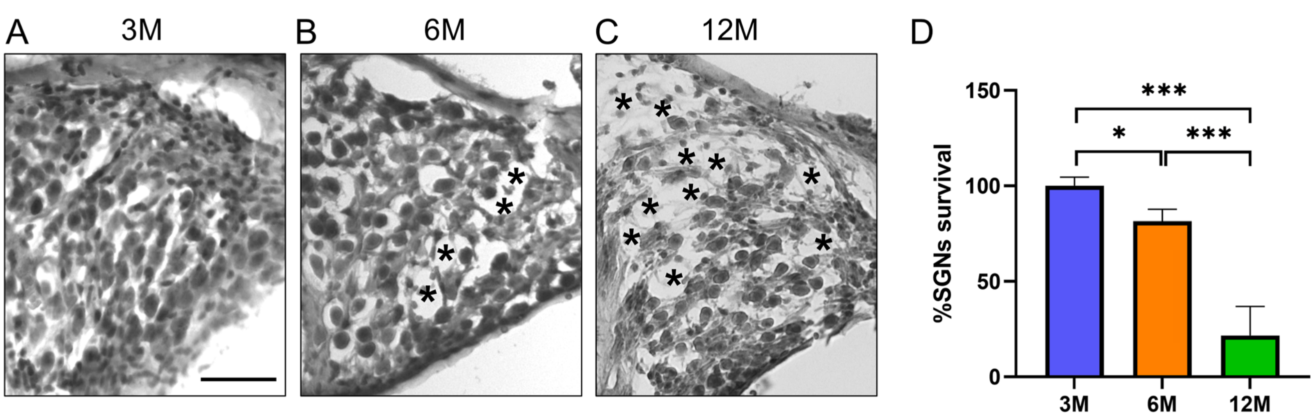

Figure 3. Age-related neuronal damage in C57BL/6 mice.
A–C: Representative images of SGN cryosections at the middle cochlear turn level. Black asterisks indicate areas with reduced neuronal profiles. D: Quantification (means ± SEM) of the relative number of SGN cell body profiles per section area in C57BL/6 mice of 3, 6, and 12 M, normalized to mice of 3 M. A progressive age-related reduction in the number of visible SGN profiles was observed in mice during physiological aging. Asterisks indicate significant differences among groups (*$p < 0.05$; ***$p < 0.001$) from one-way ANOVA. Scale bar: 50 µm.

Progressive neuronal cell loss with advancing aging

To investigate the mechanisms underlying the ARHL phenotype, we examined SGNs on cochlear cryosections stained with H&E. At 6 M, we observed a moderate but significant reduction in the number of SGN cell body profiles per section area (about 20%) compared to 3 M mice (Figure 3(A,B,D); one-way ANOVA, Tukey's *post-hoc* test, $n = 3$; $p = 0.0128$). At 12 M, the decrease in cell body profiles was more pronounced, of about 80% compared to 3 M animals

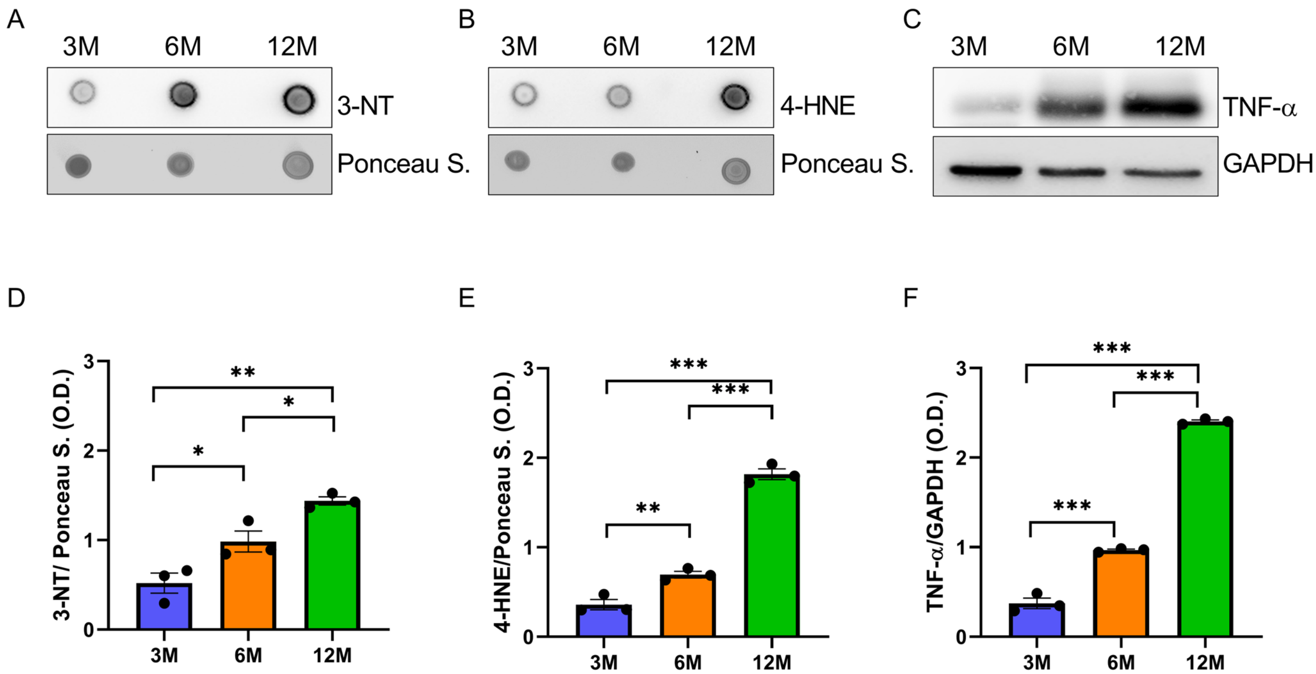

Figure 4. Increase of oxidative stress and inflammation in aging cochlea.
A, B: Representative dot blots showing a progressive increase of 3-NT and 4-HNE ($n=3$) in cochleae of mice during aging. Ponceau S staining confirmed equal protein loading. C: Western blot images showing elevated levels of TNF-α during physiological aging ($n=3$). Asterisks indicate significant differences among groups (*$p<0.05$; **$p<0.01$; ***$p<0.001$) from one-way ANOVA with repeated measures.

(Figure 3(A,C,D); one-way Anova, Tukey's *post hoc* test, $n=3$; $p<0.0001$). These results demonstrate a progressive degeneration of SGNs with age, with 12 M representing a critical stage of marked neuronal loss.

Progressive cochlear oxidative damage and inflammation in age-related cochlear degeneration

Cochlear oxidative damage is a hallmark of age-related inner ear degeneration, yet its temporal dynamics remain unclear. Using dot blot analysis, we measured markers of oxidative stress and lipid peroxidation at different ages. A significant increase in 3-NT and in 4-HNE was detected at 6 and 12 M compared to 3 M (Figure 4(A,B,D,E); 3-NT: one-way Anova, Tukey's *post hoc* test, $n=3$; 3 M *vs* 6 M $p=0.0347$; 3 M *vs* 12 M $p=0.0013$; 6 M *vs* 12 M $p=0.0381$; 4-HNE: one-way Anova, Tukey's *post hoc* test, $n=3$; 3 M *vs* 6 M $p=0.0099$; 3 M *vs* 12 M $p<0.0001$; 6 M *vs* 12 M $p<0.0001$).

Since inflammation often follows oxidative injury [5], we also examined TNF-α expression. A significant and progressive increase in TNF-α was observed at 6 and 12 M compared to 3 M (Figure 4(C,F); one-way Anova, Tukey's *post hoc* test, $n=3$; $p<0.0001$ for all comparisons).

These findings indicate that both oxidative stress and inflammation are evident in the cochlea of C57BL/6 mice starting from 6 M, when the early decline of auditory thresholds at high frequencies starts to be observed.

Discussion

This study investigated functional and molecular alterations associated with ARHL in C57BL/6 mice, focusing on the kinetics of oxidative and inflammatory damage. We found that aging is accompanied by a progressive decline in auditory function, as shown by elevated auditory thresholds, reduced neural transmission efficiency, SGN loss, and increased oxidative and inflammatory responses, all emerging at 6 M.

The progressive rise of ABR thresholds across frequencies indicated that hearing loss first affected the high-frequency range and later extended to lower frequencies, closely resembling the clinical progression of presbycusis in humans [13]. These findings reinforce the validity of C57BL/6 mice as a model of ARHL [7,8]. Genetic factors, such as the Cdh23^753A (Ahl) mutation, which affects stereocilia integrity [14], together with metabolic alterations, such as aberrant cochlear glycogen accumulation compared to CBA mice [2], contribute to early-onset ARHL in this strain, reproducing the phenotypic features described by Schuknecht [1,15].

Consistent with previous studies, ABR testing revealed elevated thresholds at high frequencies (20–32 kHz) from 6 M, progressing to profound hearing loss (>80 dB) at 12 M across all frequencies [7,8,16].

Waveform analysis revealed reduced amplitudes and increased latencies of ABR waves I and II from 6 M onward, indicating synaptic degeneration, SGN dysfunction, and impaired central processing. This functional decline is consistent with reports of cochlear synaptopathy and ribbon synapse loss as key contributors to auditory nerve dysfunction in aging [17]. These electrophysiological changes also parallel clinical difficulties in speech discrimination in noisy environments, which rely on precise and synchronous auditory processing [18]. Cochlear synaptopathy, involving early loss or dysfunction of IHC-SGN synapses, is a key contributor to auditory decline in aging and noise-induced hearing loss. These changes may precede neuronal degeneration and occur without HC loss, leading to 'hidden hearing loss.' Although in this study we do not quantify synapses between IHCs and neuronal afferent fibers, our electrophysiological data demonstrating a reduced ABR wave I amplitude and increased latency support the hypothesis of an early functional impairment at the synaptic level.

A similar temporal sequence of auditory and molecular alterations has been observed in the C57BL/6 mouse model. Progressive elevations in auditory thresholds, along with changes in ABR wave I amplitude and latency, typically emerge between 4 and 6 months of age, coinciding with the onset of synaptic dysfunction and early neuronal loss. These functional changes are accompanied by a rise in oxidative and inflammatory markers, including 4-HNE, which are recognized as early indicators of cochlear aging [7]. Consistent with these studies, our results demonstrate that both oxidative and inflammatory mechanisms become evident around 6 months, in parallel with functional alterations and preceding extensive neuronal degeneration. This supports the concept that oxidative stress and inflammation act as early, interdependent drivers of cochlear aging.

At the molecular level, elevated 3-NT and 4-HNE levels indicate nitro-oxidative stress and lipid peroxidation as major drivers of cochlear pathology from 6 M onward. Protein nitration can either reduce or enhance enzymatic activity, as shown for MnSOD, glutathione reductase, cytochrome c, and others [19], thereby amplifying dysfunction within auditory tissues. In parallel, the rise in TNF-α highlights the contribution of inflammation, supporting the view that oxidative stress and inflammation act synergistically. TNF-α promotes ROS production, creating a vicious cycle that sustains cochlear degeneration [20]. The simultaneous onset of oxidative and inflammatory markers at 6 M suggests that these processes are tightly linked and represent a pivotal turning point in ARHL. This convergence of cochlear stress, auditory nerve impairment, and central processing deficits identifies key pathogenetic mechanisms that could be targeted therapeutically.

Taken together, our findings suggest that 6 M represents a critical window for early intervention. Targeting oxidative stress and inflammation at this stage could be effective in preserving auditory function. Pharmacological modulation of these pathways holds promise for delaying or preventing cochlear degeneration and ARHL, but further studies are required to clarify the molecular cross-talk between oxidative and inflammatory signaling and to optimize therapeutic strategies and timing.

Acknowledgments

The results of this study were presented by the corresponding Author, A.R. Fetoni, at the Collegium Oto-Rhino-Laryngologicum Amicitiae Sacrum (CORLAS) 2025 meeting, held in Stockholm (August 25–27).We would like to acknowledge the contribution of the Core Facility G-STeP "Electrophysiology," Fondazione Policlinico Universitario "A. Gemelli" IRCCS. Financial support of "Ricerca Corrente " from Fondazione Policlinico Universitario "A. Gemelli" IRCCS .

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the 'Fondi INAIL BRIC id08 PAR 2022-2024'.

References

[1] Schuknecht HF. Further observations on the pathology of presbycusis. Arch Otolaryngol. 1964;80(4):369–382. doi:10.1001/archotol.1964.00750040381003.

[2] Willott JF, Parham K, Hunter KP. Comparison of the auditory sensitivity of neurons in the cochlear nucleus and inferior colliculus of young and aging C57BL/6J and CBA/J mice. Hear Res. 1991;53(1):78–94. doi:10.1016/0378-5955(91)90215-U.

[3] Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757–772. doi:10.2147/CIA.S158513.

[4] Gutteridge JM, Halliwell B. Comments on review of free radicals in biology and medicine, Second Edition, by Barry Halliwell and John M. C. Gutteridge. Free Radic. Biol Med. 1992;12:93–95. doi:10.1016/0891-5849(92)90062-l.

[5] Paciello F, Pisani A, Rolesi R, et al. Noise-induced cochlear damage involves PPAR down-regulation through the interplay between oxidative stress and inflammation. Antioxidants. 2021;10(8):1188. doi:10.3390/antiox10081188.

[6] Zuo L, Prather ER, Stetskiv M, et al. Inflammaging and oxidative stress in human diseases: from molecular mechanisms to novel treatments. Int J Mol Sci. 2019;20(18):4472. doi:10.3390/ijms20184472.

[7] Fetoni AR, Picciotti PM, Paludetti G, et al. Pathogenesis of presbycusis in animal models: a review. Exp Gerontol. 2011;46(6):413–425. doi:10.1016/j.exger.2010.12.003.

[8] Fetoni AR, Pisani A, Rolesi R, et al. Early noise-induced hearing loss accelerates presbycusis altering aging processes in the cochlea. Front Aging Neurosci. 2022;14:803973. doi:10.3389/fnagi.2022.803973.

[9] Paciello F, Pisani A, Rolesi R, et al. Oxidative stress and inflammation cause auditory system damage via glial cell activation and dysregulated expression of gap junction proteins in an experimental model of styrene-induced oto/neurotoxicity. J Neuroinflammation. 2024;21(1):4. doi:10.1186/s12974-023-02996-3.

[10] Pisani A, Rolesi R, Mohamed-Hizam V, et al. Early transtympanic administration of rhBDNF exerts a multifaceted neuroprotective effect against cisplatin-induced hearing loss. Br J Pharmacol. 2025;182(3):546–563. doi:10.1111/bph.17359.

[11] Fetoni AR, De Bartolo P, Eramo SLM, et al. Noise-induced hearing loss (NIHL) as a target of oxidative stress-mediated damage: cochlear and cortical responses after an increase in antioxidant defense. J Neurosci. 2013;33(9):4011–4023. doi:10.1523/JNEUROSCI.2282-12.2013.

[12] Fetoni AR, Paciello F, Rolesi R, et al. Styrene targets sensory and neural cochlear function through the crossroad between oxidative stress and inflammation. Free Radic Biol Med. 2021;163:31–42. doi:10.1016/j.freeradbiomed.2020.12.001.

[13] Wang J, Puel J-L. Presbycusis: an update on cochlear mechanisms and therapies. J Clin Med. 2020;9(1):218. doi:10.3390/jcm9010218.

[14] Brown SDM, Hardisty-Hughes RE, Mburu P. Quiet as a mouse: dissecting the molecular and genetic basis of hearing. Nat Rev Genet. 2008;9(4):277–290. doi:10.1038/nrg2309.

[15] Schuknecht HF, Gacek MR. Cochlear pathology in presbycusis. Ann Otol Rhinol Laryngol. 1993;102(1 Pt 2):1–16. doi:10.1177/00034894931020S101.

[16] Willott JF, Aitkin LM, McFadden SL. Plasticity of auditory cortex associated with sensorineural hearing loss in adult C57BL/6J mice. J Comp Neurol. 1993;329(3):402–411. doi:10.1002/cne.903290310.

[17] Steenken F, Heeringa AN, Beutelmann R, et al. Age-related decline in cochlear ribbon synapses and its relation to different metrics of auditory-nerve activity. Neurobiol Aging. 2021;108:133–145. doi:10.1016/j.neurobiolaging.2021.08.019.

[18] Pronk M, Deeg DJH, Festen JM, et al. Decline in older persons' ability to recognize speech in noise: the influence of demographic, health-related, environmental, and cognitive factors. Ear Hear. 2013;34(6):722–732. doi:10.1097/AUD.0b013e3182994eee.

[19] Cassina AM, Hodara R, Souza JM, et al. Cytochrome c nitration by peroxynitrite. J Biol Chem. 2000;275(28):21409–21415. doi:10.1074/jbc.M909978199.

[20] Li L, Chen D, Lin X, et al. Understanding the role of inflammation in sensorineural hearing loss: current goals and future prospects. Brain-X. 2023;1(3):e34. doi:10.1002/brx2.34.