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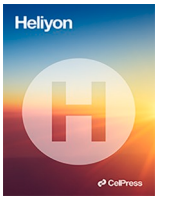

Research article

An electrophysiological early marker of age-related hearing loss in the Wistar rat model

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ABSTRACT

The goal of the present study was to determine, through a detailed study of the auditory brainstem response (ABR) waves, the possible existence of an early functional marker for the onset of presbycusis in an animal model. Toward this goal, Wistar rats were divided into four age groups: 3-month-old (3M, n = 6, control), 9-month-old (9M, n = 6), 14-month-old (14M, n = 6), and 20-month-old (20M, n = 6). ABR recordings were performed at 0.5, 1, 2, 4, 8, 16, and 32 kHz. The novel result reported here is that wave amplitudes, particularly wave II, were significantly diminished in the 9M group, even though there was no evidence of significant age-related threshold shift at that age. A significant increase in auditory thresholds with age was first detected at 14M, which further progressed at 20M, confirming our previous findings. These findings suggest that measurable alterations in ABR waves may precede age-related threshold shift and could serve as early markers to detect the onset of age-related hearing loss. Upon translation to humans, they could be used to implement early objective diagnosis, crucial to prevent or mitigate the negative consequences of presbycusis, a common, progressive, and irreversible neurodegenerative age-related disorder. This may allow, for instance, a better preservation of residual hearing, thus delaying the progression of the disease and minimizing the impact of hearing loss, ultimately improving the quality of life for those who suffer from this neurodegenerative condition.

1. Introduction

Age-related hearing loss (ARHL), or presbycusis, is a common, progressive, and irreversible neurodegenerative disorder that affects approximately between 30 and 40 % of the world's population over 60 years-old [1–3]. Besides its profound generic impact in healthy aging, it has been specifically linked to accelerated cognitive decline, impaired cognitive functioning, and higher incidence of dementia [4–9], suggesting that it is an important risk factor for AD [4–11].

It is important to consider that when the diagnosis of presbycusis is confirmed, it is because there is already a significant increase in auditory thresholds (loss of auditory sensitivity) and consequently, there will be alterations in one or more of the structures involved in

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hearing [12–15]. Unfortunately, despite the numerous scientific studies conducted and the significant efforts made in both experimental and clinical areas, there is currently no cure or treatment available for presbycusis once it begins [14,16,17]. Hence, early diagnosis and intervention of ARHL are crucial to prevent or mitigate its negative consequences.

In the study of presbycusis, animal models, such as mice and rats, have provided key information to understand this complex and multifactorial condition [12,14,18–21]. In both research and clinical practice, physiological, morphological, molecular, and genetic markers have been extensively employed for early diagnosis and monitoring of different pathologies. However, in the auditory field, although some markers have been identified in animal models [15,22,23] as well as in humans [23–27], they are not well characterized yet, and it is still unclear whether they could be used as early markers for the diagnosis of ARHL. The fact that ARHL could share common etiopathogenic pathways with other pathologies that cause or are related to hearing impairment, such as noise-induced hearing loss, drug-induced hearing loss, or even Alzheimer's disease [28–31], makes the search for possible early markers quite challenging.

Of the different methods available for diagnosing hearing loss, the most used in clinical practice and considered the gold standard is pure-tone audiometry [32–35]. On the other hand, the Auditory Brainstem Response (ABR) is the primary tool in animal models of deafness, but it is also widely used for clinical evaluations [32,35–39]. The ABR is an objective, easy to use and non-invasive technique that provides additional functional information beyond the auditory threshold, including the amplitudes and latencies of the evoked waves. Therefore, it provides critical information about both the peripheral and central auditory pathways, serving as a valuable tool for characterizing auditory alterations such as those observed in ARHL [32,35–38,40].

In the absence of specific early markers for its diagnosis, the elevation of the auditory threshold is the first indication of the presence of ARHL [1,12,15,16,26,41]. However, it is important to consider that biochemical and cellular alterations must first occur in the cochlea, which alter the auditory function, observed as increases in auditory thresholds. Therefore, it is expected that there may be a time window for earlier detection, during which some of these alterations could be reflected in the properties of ABR recordings before any significant increase in auditory thresholds. For this reason, the goal of the present study was to determine, through a detailed study of ABR waves, the possible existence of an early functional marker of the onset of presbycusis in the rat that would allow for the detection of this neurodegenerative condition even before variations in the auditory threshold arise. Besides the primary goal of providing a new tool for the early characterization of ARHL mechanisms in animal models, the results may spark the search for early markers in human ABR, which will be useful to complement standard tonal and speech audiometry.

2. Material and methods

2.1. Animals

For this study, data were collected from twenty-four male Wistar rats (Charles River, Barcelona, Spain). Only male rats were used to limit possible variations due to sex influences. The animals were kept and observed at the University of Castilla-La Mancha animal facility in Albacete, Spain, under controlled conditions. The housing environment maintained a temperature of 22–23 °C, humidity at 60 ± 5 %, and followed a 12:12-h light/dark cycle. The animals had unrestricted access to food and water. The size of the sample was determined following the principle of the four R's (replacement, reduction, refinement and responsibility) [42] and using the sample size calculator offered by Boston University in its web page (https://www.bu.edu/research/forms-policies/iacuc-sample-size-calculations/). The rats were divided, according to their age, into four groups: 3-month-old (3M, n = 6), 9-month-old (9M, n = 6), 14-month-old (14M, n = 6), and 20-month-old (20M, n = 6). The 3M group served as a control group. All experimental procedures received approval from the Ethics Committee on Animal Experimentation at the University of Castilla-La Mancha (Permit Number: PR-2019-02-05) and adhered to the regulations set forth by the European Union (Directive 2010/63/EU) and Spain (R.D. 53/2013; Law 32/2007) regarding the care and use of animals in research.

2.2. Auditory brainstem response recordings

The ABR recordings were performed as described elsewhere [12,29,30,36,43–48]. The rats were anesthetized using isoflurane inhalation (1 L/min O2 flow rate) for induction at 4 % and for maintenance at 1.5%–2%. During the experiments, the animals were placed in a sound-attenuating and electrically shielded booth (EYMASA/INCOTRON S.L., Barcelona, Spain) located inside a sound-attenuating room. The body temperature was maintained at 37.5 ± 1 °C, using a non-electrical heating pad, and monitored with a rectal probe. The responses were recorded using three subdermal needle electrodes (Rochester Electro-Medical, Tampa, FL, USA) positioned at the vertex (non-inverting), the right mastoid (inverting), and the left mastoid (ground). The BioSig System III (Tucker-Davis Technologies, Alachua, FL, USA) was utilized for stimulus presentation and auditory response recording. Calibration was performed using SigCal software (Tucker-Davis Technologies) and an ER-10B + low-noise microphone system (Etymotic Research Inc., Elk Grove, IL, USA).

Digitally generated sounds from the SigGenRP software and the RX6 Piranha Multifunction Processor hardware were transmitted to the external auditory meatus of the right ear using an EDC1 electrostatic speaker driver (Tucker-Davis Technologies) connected to an EC-1 electrostatic speaker (Tucker-Davis Technologies). As auditory stimuli, pure tone burst sounds with a 5 ms rise/fall time (without a plateau) and a $\cos^2$ envelope [12,29,30,36,43–50] were delivered at seven different frequencies (0.5, 1, 2, 4, 8, 16, and 32 kHz). Subsequently, the evoked responses were filtered within the 0.3–3.0 kHz range, averaged from five hundred waveforms, and stored for offline analysis.

2.3. Auditory brainstem response data analysis

2.3.1. Auditory thresholds

The auditory threshold was defined as the stimulus intensity that evoked auditory responses with a peak-to-peak voltage exceeding 2 standard deviations (SD) of the background activity [12,29,30,36,43–47,51]. To determine the auditory threshold at each frequency evaluated, the background activity before stimulus onset and the evoked responses were measured in 5 dB steps descending from 80 dB SPL. The maximum level of stimulus intensity was established at 80 dB to avoid any possible noise overstimulation or trauma during the recordings [12,29,30,36,43–47,51,52]. For statistical purposes, when no auditory evoked responses were obtained at 80 dB, the auditory thresholds were set at that value [12,29,30,36,43–47,51,53,54].

2.3.2. Auditory threshold shift

For each frequency studied, the threshold shift was calculated by subtracting the auditory thresholds in the different groups of age (9M, 14M and 20M) from the auditory thresholds in the 3M control rats [12,29,30,36,43–47,51].

2.3.3. Wave amplitudes

The evoked wave amplitudes were the peak-to-peak values from the positive peak to the subsequent negative trough of each one of the five evoked waves obtained during the recording [12,36,55,56].

2.3.4. The percentage of variation of the wave amplitudes

The percentage of the variation in the wave amplitudes in the older groups (9M, 14M and 20M) compared to the 3M control group were calculated for all frequencies using an enhancement index, according to the following formula [12,29]:

$$\% \text{ of variation} = [(WAO - WAC)/(WAC)] \times 100$$

Where WAO represents the wave amplitude in the older groups (9M, 14M and 20M) and WAC, the wave amplitude in the 3M control condition.

2.4. Statistical analysis

In the present study data are expressed as mean ± SEM. Measurements of ABR parameters were performed at 80 dB SPL. Comparisons between groups were performed using a one-way analysis of variance (ANOVA). If the main analysis indicated a significant effect, a Scheffé post hoc analysis was performed. The Pearson's correlation coefficient r was used to assess the statistical correlation between ABR parameters. The significance level (α) was set to 0.05, and the statistical power (β) was set at 95 %.

2.5. Preparation of figures

Excel (Microsoft Office 365) and Canvas (Deneba v6.0) software packages were used for preparation of figures in this manuscript.

3. Results

Auditory thresholds showed a significant increase with age, first detected at 14M, which progressed further in the oldest group tested at 20M. Results on auditory threshold variations with age in the Wistar rat are detailed below. They essentially confirm previously published findings [29,30,43–47,51]. The novel result reported here is that ABR wave amplitudes, specifically wave II, were

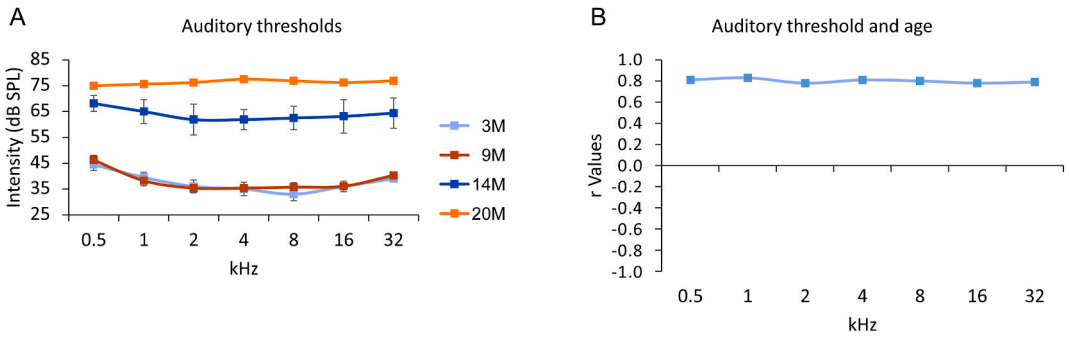

Fig. 1. Line graphs illustrating auditory thresholds and the Pearson's correlation coefficient between auditory thresholds and age at the different frequencies studied in 3M, 9M, 14M and 20M rats. (A) In both 3M and 9M animals, the mean values of the auditory thresholds were similar and consistent with those described previously. In 14M rats, the auditory thresholds were significantly higher than those found in the 3M and 9M rats. At 20M, the auditory thresholds continued to increase, being significantly higher than those at 3M, 9M and 14M animals. (B) The Pearson's correlation coefficient showed a strong positive correlation between auditory thresholds and age at all frequencies evaluated.

significantly diminished in the 9M group, at which age there was no evidence of significant age-related threshold shift. Therefore, significant changes in the amplitude of the ABR wave II precede detectable threshold shifts by several months, and therefore may constitute an early marker of age-related hearing loss in the Wistar rat model, not reported previously.

3.1. Auditory thresholds

The evaluation of the auditory thresholds in the 3M control rats demonstrated a normal pattern, comparable to that described previously in Wistar rats (Fig. 1A) [29,30,43,48,57]. Also, the ABR pattern found in the 9M group was normal and consistent with those described previously in Wistar rats of the same age (Fig. 1A) [29,30,43,48,57]. The mean auditory thresholds in this age group were like those observed in the 3M rats, with values between 35.36 ± 1.33 and 46.29 ± 1.80 dB SPL. At 14M, animals showed an evident increase in the auditory thresholds at each frequency assessed (Fig. 1A), indicating ongoing ARHL [12,29,30]. The mean values observed in this age group ranged from 61.88 ± 3.89 to 68.13 ± 3.13 dB SPL, with higher mean values at the lower frequency. By 20 months of age, auditory thresholds were further increased at all frequencies, with values similar to those reported previously for rats of the same age (Fig. 1A) [12,29,30]. Shifts in auditory thresholds confirm the increase in the mean values of auditory thresholds in the older rats. Whereas the mean value of the threshold shifts in the 9M animals, relative to the control 3M rats, ranged from −1.29 ± 0.10 to 2.71 ± 0.77 dB SPL in the older rats the mean values ranged from 23.63 ± 0.83 to 29.50 ± 2.59 dB SPL in the 14M group and from 30.50 ± 2.29 to 43.88 ± 1.18 dB SPL in the 20M animals.

The ANOVA demonstrated a significant interaction between age and auditory threshold at all frequencies (Table 1). While there were no differences in thresholds between 3M and 9M animals, the mean values obtained in the older groups were statistically significantly higher than those in the younger rats. Accordingly, the auditory thresholds at 20M were higher than those found in the 3M and 9M groups at all frequencies evaluated (Table 1), and significantly higher than the mean auditory thresholds in the 14M rats at all frequencies except for 0.5 kHz (Table 1). When compared to the younger rats (3M and 9M groups), the 14M animals also presented statistically significantly higher auditory thresholds at all tested frequencies (Table 1). An analysis using Pearson's correlation coefficient showed a strong positive correlation between auditory thresholds and age (Fig. 1B, Table 1) at all frequencies tested. Taking together, these data indicate that, as the animals age, their auditory threshold increase, confirming the time pattern of age-related hearing loss previously described in this rat strain [12,29,30].

3.2. Waveform amplitudes

Consistent with previous findings, the ABR recordings of the younger groups (3M and 9M) displayed a distinctive pattern characterized by four to five evoked waveforms following the stimulus onset (Fig. 2) [29,30,43,48,51,57]. Upon analysis, wave II was the largest, followed by waves I, IV, V and wave III, being the smallest of the waves comprising the ABR (Fig. 2). In the older animals, despite the fact of presenting a recognizable wave pattern, there was a decrease in wave amplitudes that was more evident in the 20M rats (Fig. 2). For further evaluation, the wave amplitudes (see Methods), the percentage of variation of the amplitudes (see Methods) and the Pearson's correlation coefficient were calculated for each wave as function of all frequencies assessed in all groups of age.

Regarding wave I, when the mean amplitudes were plotted as function of frequency (Fig. 3A), it was evident that the values of the oldest rats (20M) were the lowest of all groups, with no apparent differences among 3M, 9M and 14M animals. The ANOVA showed a significant interaction between age and wave amplitudes (Table 2), confirming the absence of differences between the younger rats (3M and 9M) and demonstrating a significant reduction in the values in the 20M group compared to the 3M, 9M and 14M animal groups at all frequencies evaluated (Fig. 3A. Statistical power was higher than 99 % for all cases. However, there was also a significant difference when the 14M group was compared with the 3M and 9M rats, but only at 16 ($p < 0.05$) and 32 ($p < 0.05$) kHz. These differences were clearer when the percentage of variation in the wave amplitudes (see Methods), was calculated. Whereas mean values

Table 1
Analysis of the interaction between age and auditory thresholds.

ANOVA							
	0.5 kHz	1 kHz	2 kHz	4 kHz	8 kHz	16 kHz	32 kHz
$F_{(3,36)} = p$	54.23 ***	52.88 ***	44.79 ***	81.69 ***	64.86 ***	46.48 ***	57.82 ***
Significance levels							
3M vs 9M	NS	NS	NS	NS	NS	NS	NS
3M vs 14M	***	***	***	***	***	***	***
3M vs 20M	***	***	***	***	***	***	***
9M vs 14M	***	***	***	***	***	***	***
9M vs 20M	***	***	***	***	***	***	***
14M vs 20M	NS	*	*	**	*	*	*
Pearson's Correlation Coefficient							
	0.5 kHz	**1 kHz**	**2 kHz**	**4 kHz**	**8 kHz**	**16 kHz**	**32 kHz**
$r_{(38)} = p$	0.81 ***	0.83 ***	0.78 ***	0.81 ***	0.80 ***	0.78 ***	0.79 ***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS No significant differences.

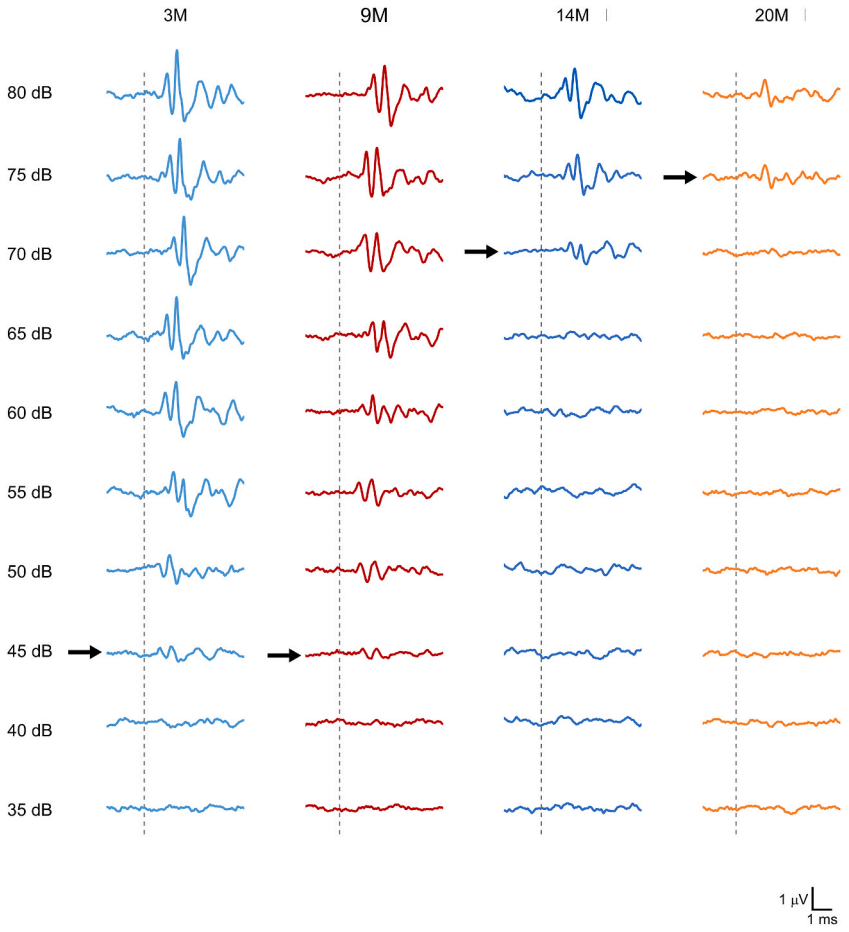

Fig. 2. Examples of ABR waveforms recordings during 10 ms to 0.5 kHz tone burst sounds, from representative 3M, 9M, 14M and 20M rats. Interval was increased in 5 dB steps. Dashed line indicates the stimulus onset, and the arrows show the auditory threshold. The rats in the 3M group exhibit the characteristic wave pattern, consisting of 4–5 waves evoked after the stimulus onset, which decrease in amplitude as the stimulus intensity decreases. The auditory threshold was determined at 45 dB SPL (arrow). In 9M, 14M and 20M rats, there was an age-related decrease in wave amplitudes compared to that in the 3M animals. However, while in 9M animals the auditory threshold was similar to that seen in 3M rats, in 14M and 20M rats the recordings showed an elevation of auditory thresholds at 70 and 75 dB, respectively.

in the 9M rats ranged between -4.97 ± 0.14 and 3.68 ± 0.73 % close to the mean values of the 3M rats, mean values in the 14M animals were lower, between -11.67 ± 7.67 and -38.28 ± 7.86 % relative to the younger rats and were even lower in the 20M animals with mean values between -49.90 ± 23.42 and -87.46 ± 20.87 % regarding 3M rats. There is an evident reduction, greater at higher frequencies, in wave amplitudes as the age of the animals increases, indicating an inverse relationship between amplitude and age. To confirm this observation, the possible correlation between wave I amplitude and the main variables used in this study, age, and auditory thresholds, were evaluated by means of Pearson's correlation coefficient. The results showed that there was a significant inverse correlation in most of the frequencies evaluated, except for 4 kHz, between wave I amplitude and age (Fig. 3B, Table 2) and between wave I amplitude and auditory thresholds (Fig. 3C, Table 2).

For wave II, when the mean amplitudes of all groups were plotted as a function of frequencies (Fig. 4A), a consistent decrease in wave amplitudes was observed across all evaluated frequencies in rats at 9, 14, and 20 months compared to the 3-month-old animals. The ANOVA analysis revealed an interaction between wave II amplitudes and age (Table 2), confirming that the mean amplitude values in the oldest animals (20 months) were significantly smaller than those in the 3 ($p < 0.001$ at all frequencies), 9 ($p < 0.001$ at all frequencies), and 14 ($p < 0.01$ at low and medium frequencies, $p < 0.05$ at high frequencies) months-old rats. In the 14-month-old rats, the values were significantly smaller at all frequencies ($p < 0.01$) compared to the 3-month-old group, and at 32 kHz ($p < 0.05$) compared to the 9-month-old group. Finally, in the 9-month-old rats, the mean values were significantly smaller than those in the 3-month-old animals at 0.5 ($p < 0.05$), 1 ($p < 0.05$), 2 ($p < 0.05$), 4 ($p < 0.05$), 8 ($p < 0.05$), and 16 kHz ($p < 0.05$), but not at 32 kHz (Fig. 4A). The percentage of variation in the mean amplitudes of wave II in older rats relative to the 3-month-olds demonstrated the magnitude of the progressive reduction in wave amplitudes as the animals aged. Specifically, the reduction exceeded 25 % at 9 months (ranging from 25.94 % ± 12.30 %–33.51 % ± 11.78 %), 40 % at 14 months (ranging from 40.39 % ± 19.14 %–56.18 % ± 18.06 %), and 70 % at 20 months (ranging from 71.50 % ± 24.95 %–95.11 % ± 20.35 %). The Pearson's correlation coefficient showed a strong

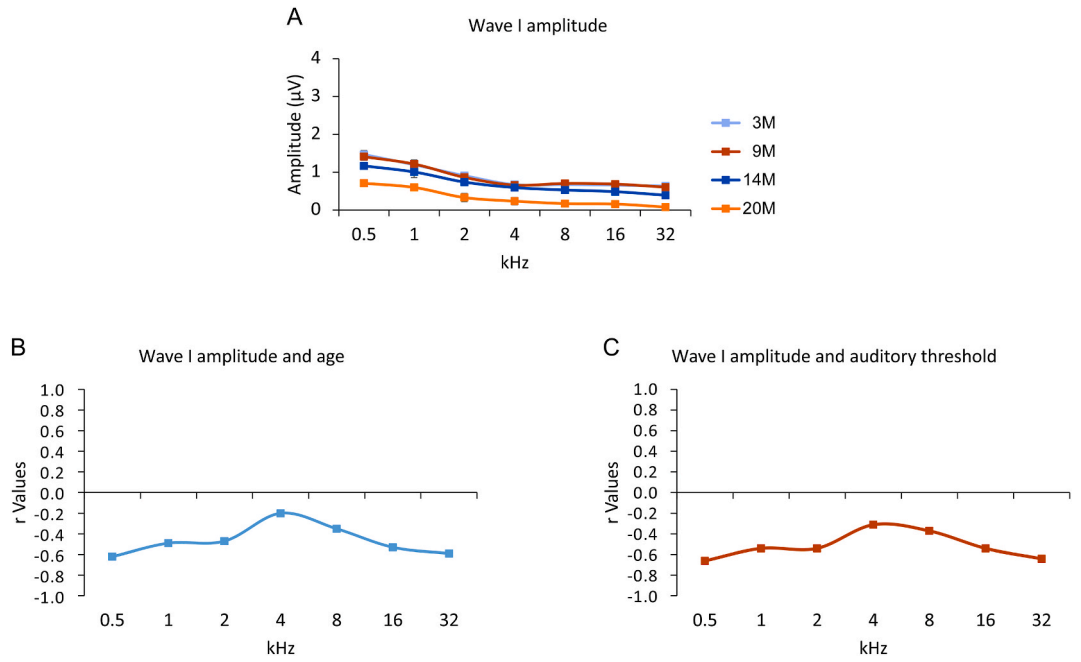

Fig. 3. Line graphs illustrating wave I amplitudes (in μV), as a function of the frequencies evaluated in animals at 3, 9, 14 and 20 months of age. (A) The mean values at 3M, 9M and 14M animals were apparently similar without significant differences among them, while at 20M there was an evident decrease in the mean amplitude values compared to the younger rats, indicating an age-related effect at least for 14- and 20-months rats. (B, C) The Pearson's correlation coefficient revealed a significant inverse correlation at all frequencies, except for 4 kHz, between wave I amplitude and age (B) and between wave I amplitude and auditory thresholds (C).

Table 2
Analysis of the interaction between wave amplitudes, age and auditory thresholds.

ANOVA of the interaction between wave amplitudes and age															
WAVES		0.5 kHz		1 kHz		2 kHz		4 kHz		8 kHz		16 kHz		32 kHz	
I	$F_{(3,36)}$ =	11.04	***	5.68	***	10.01	***	5.28	**	11.38	***	12.28	***	16.21	***
II	$F_{(3,36)}$ =	28.97	***	14.31	***	18.92	***	15.94	***	18.41	***	21.59	***	20.82	***
III	$F_{(3,36)}$ =	2.57	NS	1.37	NS	1.56	NS	0.70	NS	6.46	**	4.38	**	7.08	***
IV	$F_{(3,36)}$ =	6.37	**	5.37	**	3.54	*	6.66	**	6.01	**	5.14	**	4.74	**
V	$F_{(3,36)}$ =	23.05	***	13.08	***	15.29	***	9.48	***	9.42	***	12.69	***	8.43	***
Pearson's Correlation Coefficient of the interaction between wave amplitudes and age															
WAVES		**0.5 kHz**		**1 kHz**		**2 kHz**		**4 kHz**		**8 kHz**		**16 kHz**		**32 kHz**	
I	$r_{(38)}$ =	−0.62	***	−0.49	**	−0.47	**	−0.20	NS	−0.35	*	−0.53	**	−0.59	***
II	$r_{(38)}$ =	−0.81	***	−0.73	***	−0.68	***	−0.65	***	−0.66	***	−0.74	***	−0.73	***
III	$r_{(38)}$ =	−0.27	NS	−0.20	NS	−0.13	NS	−0.13	NS	−0.39	*	−0.39	*	−0.41	*
IV	$r_{(38)}$ =	−0.50	***	−0.51	***	−0.32	*	−0.43	**	−0.32	*	−0.44	**	−0.37	*
V	$r_{(38)}$ =	−0.76	***	−0.68	***	−0.56	***	−0.50	**	−0.42	*	−0.61	***	−0.54	***
Pearson's Correlation Coefficient of the interaction between wave amplitudes and auditory thresholds															
WAVES		**0.5 kHz**		**1 kHz**		**2 kHz**		**4 kHz**		**8 kHz**		**16 kHz**		**32 kHz**	
I	$r_{(38)}$ =	−0.66	***	−0.54	***	−0.54	***	−0.31	NS	−0.37	*	−0.54	***	−0.64	***
II	$r_{(38)}$ =	−0.71	***	−0.60	***	−0.67	***	−0.66	***	−0.56	***	−0.70	***	−0.72	***
III	$r_{(38)}$ =	−0.24	NS	−0.28	NS	−0.30	NS	−0.19	NS	−0.19	NS	−0.32	*	−0.40	*
IV	$r_{(38)}$ =	−0.52	***	−0.49	**	−0.50	**	−0.51	**	−0.54	***	−0.54	***	−0.49	**
V	$r_{(38)}$ =	−0.60	***	−0.59	***	−0.58	***	−0.50	**	−0.47	**	−0.63	***	−0.52	**

*p < 0.05; **p < 0.01; ***p < 0.001; NS No significant differences

negative correlation between wave II amplitudes and age (Fig. 4B, Table 2), as well as auditory thresholds (Fig. 4C, Table 2) at all evaluated frequencies, with p < 0.001 in all cases.

In Wistar rats, wave III is the smallest and most irregular of the five that comprise the ABR recordings. Nevertheless, there was also a discernible effect of age on the amplitude of wave III when mean amplitudes were plotted as a function of frequency (Fig. 5A). The ANOVA test indicated that this effect was primarily observed at higher frequencies (Table 2) and in older animals (14 months and 20

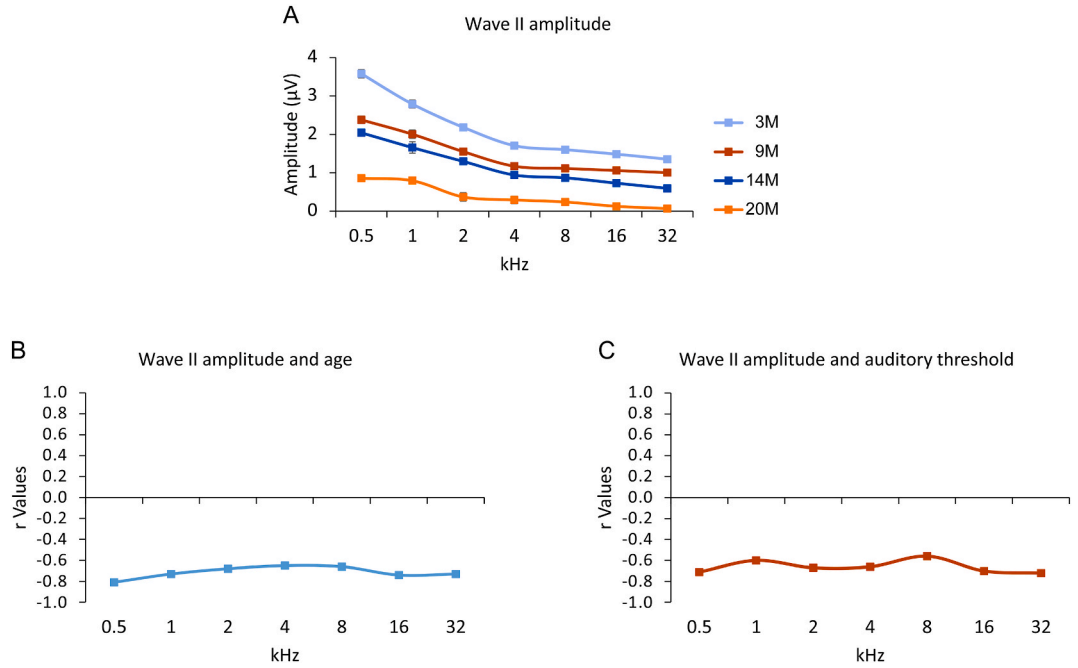

Fig. 4. Line graphs illustrating wave II amplitudes (in μV), as a function of the frequencies evaluated in rats at 3, 9, 14 and 20 months of age. (A) The figure shows a consistent age-related reduction in mean wave amplitudes at all evaluated frequencies in 9M, 14M and 20M rats compared to that in 3M animals. (B, C) The Pearson's correlation coefficient revealed a strong inverse correlation at all frequencies between wave II amplitude and age (B) and between wave II amplitude and auditory thresholds (C).

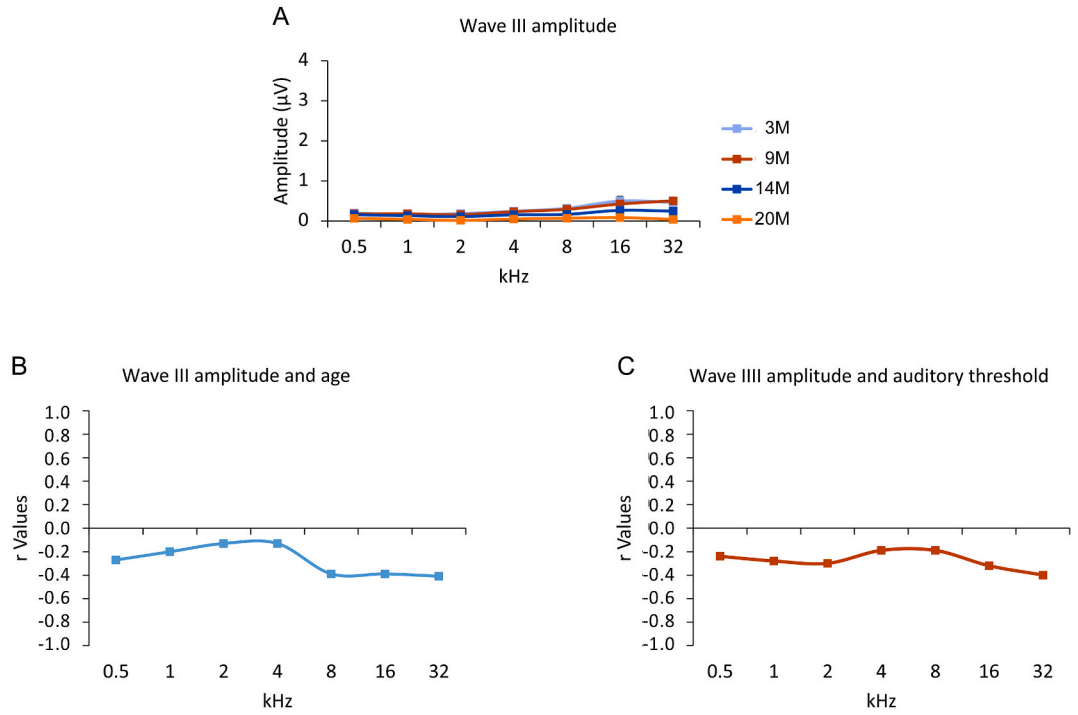

Fig. 5. Line graphs illustrating wave III amplitudes (in μV) as a function of the frequencies evaluated in 3M, 9M, 14M and 20M animals. (A) Even though wave III is the most irregular and small of all waves in rats, there was an age-related effect in the mean amplitude values in 14M and 20M animals, particularly at the higher frequencies compared to that at 3M and 9M. (B, C) The Pearson's correlation coefficient showed a weak inverse correlation for the higher frequencies, between wave III amplitude and age (B) and between wave III amplitude and auditory thresholds (C).

months). Meanwhile, no significant differences were observed between the 3 and 9-month-old groups, or between the 14 and 20-month-old rats. The mean amplitude values of wave III in the older rats (14 months and 20 months) were significantly lower than those in the younger animals (3 months and 9 months) at 8, 16, and 32 kHz ($p < 0.05$). The percentage of variation in amplitudes (Fig. 5B) ranged from −14.57 % ± 0.94 %–13.21 % ± 2.83 % in the 9-month-old animals. In older animals, the reduction was more pronounced at lower frequencies, ranging from −16.67 % ± 16.25 % to −47.50 % ± 14.22 % at 14 months and from −66.25 % ± 29.89 % to −91.22 % ± 26.16 % at 20 months. Pearson's correlation coefficient demonstrated a weak inverse correlation between the wave III amplitudes and age (Fig. 5B, Table 2), as well as the auditory thresholds (Fig. 5C, Table 2), but only at the higher frequencies evaluated. For instance, there was a weak negative correlation between the amplitude of wave III and age at 8 kHz ($r = -0.39$, $p < 0.05$), 16 kHz ($r = -0.39$, $p < 0.05$), and 32 kHz ($r = -0.41$, $p < 0.05$), as well as with auditory thresholds at 16 kHz ($r = -0.32$, $p < 0.05$) and 32 kHz ($r = -0.40$, $p < 0.05$) (Fig. 5C and D, Table 2).

In the context of wave IV, as shown in Fig. 6A which depicts mean amplitudes as a function of frequency, there was a decrease in amplitudes, particularly in the oldest animals (20 months), when compared to the 3-, 9-, and 14-month-old-animals. Further analysis using ANOVA demonstrated a significant interaction between amplitude and age (Table 2), confirming that mean amplitude values of wave IV in the 20-month-old animals were significantly smaller than those observed at 3 ($p < 0.01$ at all frequencies) and 9 ($p < 0.01$ at 0.5, 1, 4, 8, and 32 kHz, and $p < 0.05$ at 2 and 16 kHz) month-old animals. Compared to the 14-month-old group, the 20-month-olds were also significantly smaller at 0.5 kHz ($p < 0.05$) and 1 kHz ($p < 0.05$), without differences observed at the remaining frequencies. No other significant differences were observed when comparing the 14-month-old rats with the 3- and 9-month-olds, or between the 3- and 9-month-olds. Regarding the percentage of variation in wave IV amplitude, in the 9-month-old group, the values ranged from −13.99 % ± 1.47 %–8.35 % ± 0.06 %, showing no significant differences when compared to the 3-month-old rats. In the 14-month-old group, although no significant differences were observed compared to younger rats, the mean amplitudes were reduced, ranging from −3.85 % ± 12.19 % to −44.47 % ± 9.43 %. Finally, in the 20-month-old rats, the percentage of reduction in the amplitudes was more evident, with values ranging from −65.95 % ± 27.83 % to −96.89 % ± 15.73 % relative to the 3-month-olds. The evaluation of the correlation between wave IV amplitude and age or auditory thresholds using Pearson's correlation coefficient demonstrated a moderate but significant negative correlation in age (Fig. 6B, Table 2) and auditory thresholds (Fig. 6C, Table 2), confirming the inverse correlation between these two parameters of the ABRs.

Regarding wave V, the mean amplitudes plotted as a function of the studied frequencies also revealed a significant age-related effect on the amplitude of this wave. This effect was particularly pronounced in the 20M group (Fig. 7A), with no discernible differences between the 3M and 9M animals. The 14M rats exhibited similarities to the younger rats in the low and medium frequencies but showed a more substantial reduction in the higher frequencies (7A). An ANOVA test confirmed a statistically significant interaction between wave V amplitude and age (Table 2). Further analysis using post-hoc tests revealed that the 20M had the smallest mean

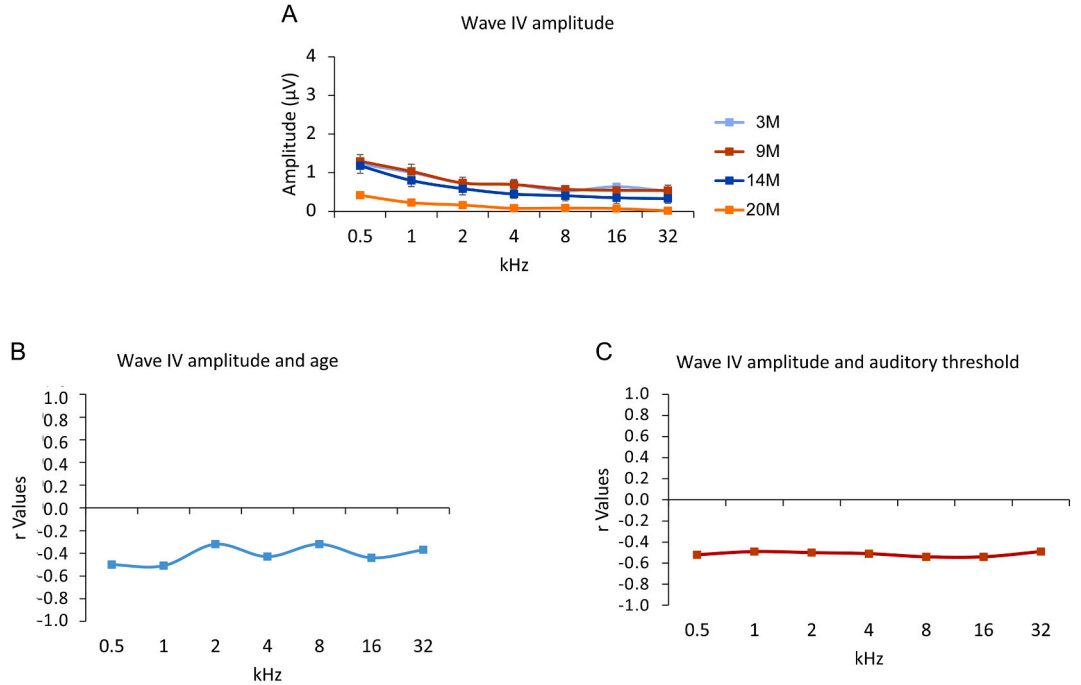

Fig. 6. Line graphs illustrating wave IV amplitudes (in μV), as a function of the frequencies evaluated in animals at 3, 9, 14 and 20 months of age. (A) Similar to wave I amplitude, the mean amplitude values at 3M, 9M and 14M rats were similar, while at 20M there was an age-related decrease in the mean amplitude values compared to the younger rats. (B, C) The Pearson's correlation coefficient revealed a moderate significant inverse correlation at all frequencies, between wave IV amplitude and age (B) and between wave IV amplitude and auditory threshold (C).

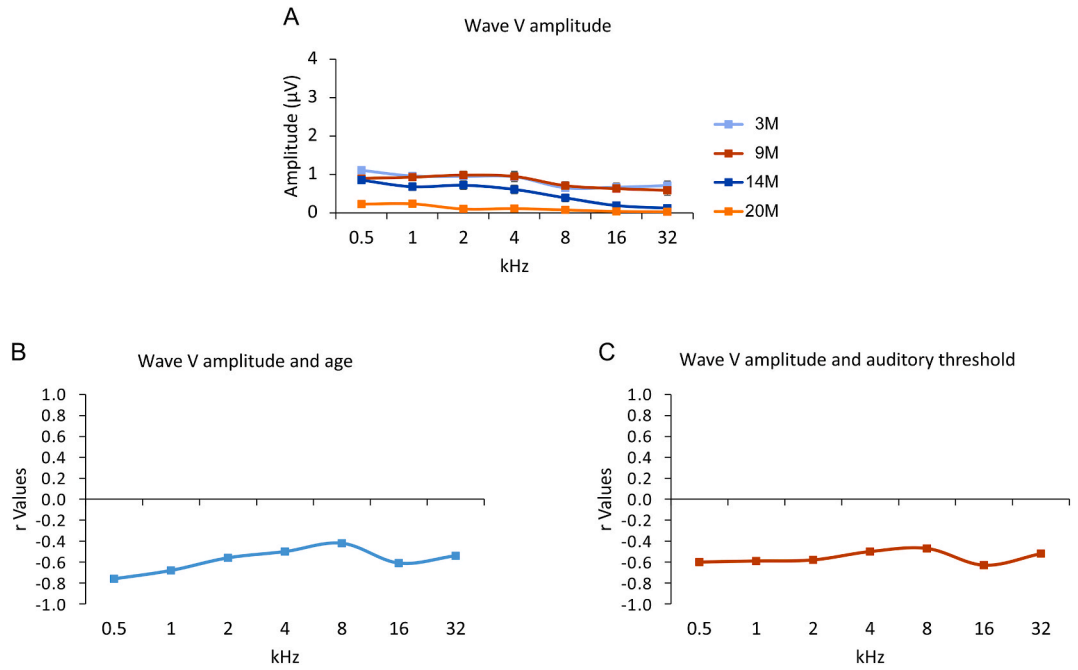

Fig. 7. Line graphs illustrating wave V amplitudes (in μV) as a function of the frequencies evaluated at 3M, 9M, 14M and 20M animals. (A) There were no noticeable differences between mean values in the 3M and 9M rats. At 14M, there was an age-related reduction in the mean amplitude values for the higher frequencies compared to the 3M and 9M rats. However, the age-relate decrease was particularly evident at all frequencies at 20M when compared to the other animal groups. (B, C) The Pearson's correlation coefficient showed a moderate to strong inverse correlation at all frequencies between wave V amplitude and age (B) and between wave V amplitude and auditory threshold (C).

amplitude values for wave V compared to the 3M ($p < 0.01$ for all frequencies) and 9M ($p < 0.01$ for all frequencies) rats. Additionally, at 0.5 kHz ($p < 0.05$), 1 kHz ($p < 0.05$), and 2 kHz ($p < 0.05$), the 20M rats had significantly smaller mean amplitudes compared to the 14M rats. Furthermore, the 14M rats exhibited reduced mean amplitudes at 16 kHz and 32 kHz compared to the 3M ($p < 0.01$) and 9M ($p < 0.01$) animals. Notably, no significant differences were found between the 3M and 9M rats. The percentage of variation relative to the 3M control group indicated that the 9M rats closely approximated the 3M values, ranging from -19.22 ± 0.84 % to 8.73 ± 2.74 %. In contrast, the 14M rats exhibited greater variability, with mean values ranging from -23.17 ± 19.95 % in the low and medium frequencies to -82.34 ± 15.15 % in the higher frequencies. Like other waveforms, the reduction in wave V amplitude at 20 months was more pronounced, with mean values ranging from -75.10 ± 27.33 % to -95.94 ± 17.91 %. Pearson's correlation coefficient demonstrated a moderate to strong negative correlation between wave V amplitude and age (Fig. 7B, Table 2), as well as between wave V amplitude and auditory thresholds (Fig. 7C, Table 2).

4. Discussion

The results obtained in the present study demonstrate that, as Wistar rats age, there are alterations in the evoked waves obtained in the ABR even before the first significant auditory threshold shift can be detected. Specifically, a decrease in the amplitude of all evoked waves was observed, which was more evident in wave II, with a strong inverse correlation with both age and auditory threshold, at all evaluated frequencies. The fact that there are decreases in the amplitudes of the ABR waves prior to the increase in the auditory threshold, which is the hallmark of presbycusis [1,12,15,16,26,41], indicates that, at least in the Wistar rat model, there are electrophysiological events, both in the auditory periphery and central pathways, which are recordable significantly earlier than aging-related auditory threshold shifts, and which may anticipate symptomatic ARHL. If comparable early functional alterations are found in the huma clinic, with the appropriate techniques, they could serve as early markers to anticipate onset of ARHL and can be used to implement preventative and future therapeutic strategies earlier than they are currently applied.

The ABR is a recording technique that measures the electrical activity of the brainstem in response to sound stimulation. Typically, in humans, it consists of 5–7 evoked waves that appear within the first 10 ms after the stimulus [32,36,58–60]. This widely used and well-characterized technique provides functional information beyond the objective auditory threshold of the subject under study. It also includes additional details about the amplitudes and latencies of these evoked waves, which could help localize possible peripheral and/or central auditory alterations [32,36,58–60]. The ABR is an indispensable tool for studying animal models of auditory pathologies and it is highly valuable in clinical practice. For instance, ABRs have provided significant ground information for the study of ARHL in rodent models, including both mice and rats, which has contributed to elucidating etiopathogenic aspects of this disease [12,14,19,20,29,30,51,61–63].

The analysis of data obtained in the present study, using electrophysiological ABR recordings in an animal model of presbycusis, the Wistar rat, confirms that, like in humans, there is a gradual and irreversible increase in auditory thresholds as the animal ages. As previously described in Wistar rats and Fischer 344 rats [12,20,29,30,51], this increase is already evident at 12–14 months of age and persists and increases until at least 20 months. The alteration in the auditory function arises from underlying morphological changes within the auditory system, particularly affecting the cochlea. With advancing age, there is a progressive loss of hair cells, alterations in the structure of the stria vascularis, and a reduction in the number of fibrocytes within the spiral ligament. These changes, whether occurring individually or collectively, significantly contribute to the hearing loss observed in presbycusis [13–16,20,26,51,64,65]. The loss of hair cells leads to a decrease in excitatory afferent transmission from the cochlea to the spiral ganglion. This, combined with alterations in the endocochlear potential due to damage in the stria vascularis and the spiral ligament, and a reduction in synaptic efficacy in central auditory nuclei, results in impaired signal propagation along the entire auditory pathway [12,64,66–69]. This leads to diminished evoked responses from the structures comprising the auditory pathway, and a decrease in the amplitude of the waves recorded in the ABR [12,29,65,70,71]. In the specific case of wave II in the Wistar rat, it has been shown that in the cochlear nuclei, the generator of this wave [36,72–74], there is a concomitant decrease in both excitatory and inhibitory synaptic vesicular transporters suggesting altered synaptic efficacy that will be reflected in decreases in wave II amplitude [12]. It is important to note that there are differences in wave generators between rats and humans [36,59,72,73]. Consequently, although both exhibit an ABR pattern consisting of 4–5 waves, in rats, the dominant wave is wave II, as previously indicated, whereas in humans, the dominant waves are V and III [59]. In fact, wave V is the most used for electrophysiological evaluations in humans. These differences should be considered when extrapolating these results, as changes in humans are likely to be more discernible in waves V and III than in wave II.

Our findings also demonstrate a reduction in the amplitude of all evoked waves with age which is consistent with results in humans [26,60,65,71] and other animal models including Fischer 344 rats, mice, guinea pigs and cats [14,70,71,75]. In Wistar rats, the significant reductions in wave amplitudes have been observed between 12 and 14 months becoming even more pronounced between 18 and 20 months [12,29,30]. Considering that the objective measurement of auditory threshold is based on the relationship between the amplitudes of the largest evoked wave and the baseline noise recorded in the ABR, hearing loss is determined when there are no significant differences between these two amplitudes at a specific frequency and intensity [12,29,30,36,43–47,51]. Given that this reduction in wave amplitude should be gradual and progressive, unless it is accelerated due to conditions such as vascular pathologies, noise overexposure, or the use of ototoxic medication, it would be logical to expect that significant reductions in wave amplitude could be observed without significant changes in auditory threshold. Unfortunately, there are no detailed studies in the Wistar rat strain on the wave amplitudes at intermediate age when hearing is apparently normal without evidence of significant age-related threshold shift. For that reason, in this study the electrophysiological hearing evaluation was conducted at 9 months, which falls between the group of 3–6 months old rats, when auditory threshold and wave amplitude values are normal, and that of 12–14 months, during which these values are clearly altered in Wistar rats [12,29,30]. The data demonstrated that, when compared to younger animals, there is a significant decrease in all recorded ABR waveforms across all studied frequencies, while auditory thresholds at that age were not significantly different from those found in younger rats. These findings suggest the presence of cochlear alterations months before significant increases in auditory threshold are detected. These alterations may be substantial enough to impact the synaptic efficacy of the system, as evidenced by a significant decrease in the amplitude of the ABR waveforms. However, they are not sufficient to significantly raise the auditory threshold in rats. It is important to note that, although latencies are an additional parameter provided by the ABR recordings, they cannot be used as potential early electrophysiological markers of presbycusis. As previously described, latencies in Wistar rats are altered beyond 14 months, and only at the medium and high frequencies of waves IV and V [12].

That period during which significant alterations in the amplitudes of evoked waves can be observed without significant loss of auditory threshold can be considered a “critical early window” for detection of presbycusis. This critical early window serves the important purpose of using this electrophysiological parameter as an early indicator of ARHL in experimental studies in animal models. In addition, if such window is found in humans it should enable the application of early tests to implement therapeutic and preventive strategies on risk factors [15,26,64], long before open manifestations of the disease lead to diagnosis based on increase in the auditory threshold. It is reasonable to expect that any preventative/therapeutic interventions carried out during the initial stages of presbycusis, when cochlear alterations do not significantly impact hearing, will yield far greater benefits than those performed when hearing is already severely affected.

Despite growing evidence of the impact of presbycusis on public health costs and several studies highlighting the cost-effectiveness of implementing screening for hearing loss detection, especially in individuals over 50 [76–78], there are currently no early detection programs for this condition worldwide. Many individuals with ARHL remain unaware of their condition or delay seeking medical assistance due to stigma, lack of awareness, or limited access to healthcare services. Unfortunately, professional help is often sought only when the affected person or a family member notices the hearing loss, by which time presbycusis is already present. The gold standard for diagnosing hearing loss in most adults is pure-tone audiometry [32–34,79]. This quick and easy test generates a graph of auditory thresholds for each frequency studied, indicating where there may be a decrease in hearing [32–34,79]. Despite its usefulness, pure-tone audiometry has limitations, such as being a subjective test that requires patient cooperation and not providing enough information about central auditory problems. Other tests, like auditory effort tests and central auditory processing tests, can also detect early hearing alterations [80–84] but are similarly subjective. Ideally, these tests should be complemented by more objective, easy-to-use, and non-invasive assessments, such as ABR test [32,36,58–60]. While considerable progress has been made in studying presbycusis, our mechanistic understanding remains limited. Animal models are extremely valuable for unraveling etiopathogenic mechanisms and allowing for quicker evaluations, which would take much longer in humans. The use of animal models has significantly contributed to a better understanding of the effect of ARHL on auditory function, primarily through the study of variations in evoked responses in ABRs according to age. The results of these studies could spark the search for similar markers in humans, helping

to overcome the limitations of tonal audiometry.

CRediT authorship contribution statement

Juan Carlos Alvarado: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Verónica Fuentes-Santamaría:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Zaskya Benítez-Maicán:** Methodology, Investigation. **Carmen María Díaz García:** Methodology, Investigation. **María Cruz Gabaldón Ull:** Methodology, Investigation. **José M. Juiz:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This work has not been published previously, and it is not under consideration for publication elsewhere, and its publication is approved by all authors. Also, the authors declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] G.A. Gates, J.H. Mills, Presbycusis, Lancet 366 (2005) 1111–1120, https://doi.org/10.1016/S0140-6736(05)67423-5.
[2] B. Shield, Evaluation of the social and economic costs of hearing impairment, Hear-It AISBL. http://www.french.press.hear-it.org/multimedia/hear_it_report_october_2006.pdf, 2006. (Accessed 14 October 2015).
[3] World Health Organization, World Report on Hearing, World Health Organization, Geneva, Switzerland, 2021. https://www.who.int/publications/i/item/world-report-on-hearing.
[4] A. Chern, J.S. Golub, Age-related hearing loss and dementia, Alzheimer Dis. Assoc. Disord. 33 (2019) 285–290, https://doi.org/10.1097/WAD.0000000000000325.
[5] D. Llano, L. Issa, P. Devanarayan, V. Devanarayan, Alzheimer's disease neuroimaging initiative (ADNI), hearing loss in Alzheimer's disease is associated with altered serum lipidomic biomarker profiles, Cells 9 (2020) 2556, https://doi.org/10.3390/cells9122556.
[6] D.G. Loughrey, M.E. Kelly, G.A. Kelley, S. Brennan, B.A. Lawlor, Association of age-related hearing loss with cognitive function, cognitive impairment, and dementia: a systematic review and meta-analysis, JAMA Otolaryngol. Neck Surg. 144 (2018) 115, https://doi.org/10.1001/jamaoto.2017.2513.
[7] F. Panza, M. Lozupone, V. Solfrizzi, R. Stallone, A. Bellomo, A. Greco, A. Daniele, D. Seripa, G. Logroscino, Cognitive frailty: a potential target for secondary prevention of dementia, Expet Opin. Drug Metabol. Toxicol. 13 (2017) 1023–1027, https://doi.org/10.1080/17425255.2017.1372424.
[8] A. Shan, F.R. Lin, C.L. Nieman, Age-related hearing loss: recent developments in approaching a public health challenge, Curr. Otorhinolaryngol. Rep 8 (2020) 24–33, https://doi.org/10.1007/s40136-020-00271-0.
[9] G. Livingston, J. Huntley, K.Y. Liu, S.G. Costafreda, G. Selbæk, S. Alladi, D. Ames, S. Banerjee, A. Burns, C. Brayne, N.C. Fox, C.P. Ferri, L.N. Gitlin, R. Howard, H.C. Kales, M. Kivimäki, E.B. Larson, N. Nakasujja, K. Rockwood, Q. Samus, K. Shirai, A. Singh-Manoux, L.S. Schneider, S. Walsh, Y. Yao, A. Sommerlad, N. Mukadam, Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission, Lancet 404 (2024) 572–628, https://doi.org/10.1016/S0140-6736(24)01296-0.
[10] R.S. Thomson, P. Auduong, A.T. Miller, R.K. Gurgel, Hearing loss as a risk factor for dementia: a systematic review: hearing Loss and Dementia Systematic Review, Laryngoscope Investig. Otolaryngol. 2 (2017) 69–79, https://doi.org/10.1002/lio2.65.
[11] M. Zheng, J. Yan, W. Hao, Y. Ren, M. Zhou, Y. Wang, K. Wang, Worsening hearing was associated with higher β-amyloid and tau burden in age-related hearing loss, Sci. Rep. 12 (2022) 10493, https://doi.org/10.1038/s41598-022-14466-6.
[12] J.C. Alvarado, V. Fuentes-Santamaría, M.C. Gabaldón-Ull, J.L. Blanco, J.M. Juiz, Wistar rats: a forgotten model of age-related hearing loss, Front. Aging Neurosci. 6 (2014), https://doi.org/10.3389/fnagi.2014.00029.
[13] T.H. Chisolm, J.F. Willott, J.J. Lister, The aging auditory system: anatomic and physiologic changes and implications for rehabilitation, Int. J. Audiol. 42 (Suppl 2) (2003), 2S3-10.
[14] A.R. Fetoni, P.M. Picciotti, G. Paludetti, D. Troiani, Pathogenesis of presbycusis in animal models: a review, Exp. Gerontol. 46 (2011) 413–425, https://doi.org/10.1016/j.exger.2010.12.003.
[15] J. Wang, J.-L. Puel, Presbycusis: an update on cochlear mechanisms and therapies, J. Clin. Med. 9 (2020) 218, https://doi.org/10.3390/jcm9010218.
[16] Q. Huang, J. Tang, Age-related hearing loss or presbycusis, Eur. Arch. Oto-Rhino-Laryngol. 267 (2010) 1179–1191, https://doi.org/10.1007/s00405-010-1270-7.
[17] T. Yamasoba, F.R. Lin, S. Someya, A. Kashio, T. Sakamoto, K. Kondo, Current concepts in age-related hearing loss: epidemiology and mechanistic pathways, Hear. Res. 303 (2013) 30–38, 10/gcv646.
[18] E.C. Bielefeld, D. Coling, G.-D. Chen, M. Li, C. Tanaka, B.-H. Hu, D. Henderson, Age-related hearing loss in the Fischer 344/NHsd rat substrain, Hear. Res. 241 (2008) 26–33, https://doi.org/10.1016/j.heares.2008.04.006.

[19] M.R. Bowl, S.J. Dawson, The mouse as a model for age-related hearing loss - a mini-review, Gerontology 61 (2014) 149–157, https://doi.org/10.1159/000368399.

[20] D. Buckiova, J. Popelar, J. Syka, Aging cochleas in the F344 rat: morphological and functional changes, Exp. Gerontol. 42 (2007) 629–638, https://doi.org/10.1016/j.exger.2007.02.007.

[21] J. Syka, The Fischer 344 rat as a model of presbycusis, Hear. Res. 264 (2010) 70–78, https://doi.org/10.1016/j.heares.2009.11.003.

[22] Q. Ruan, C. Ma, R. Zhang, Z. Yu, Current status of auditory aging and anti-aging research: auditory aging and anti-aging, Geriatr. Gerontol. Int. 14 (2014) 40–53, https://doi.org/10.1111/ggi.12124.

[23] N. Watson, B. Ding, X. Zhu, R.D. Frisina, Chronic inflammation – inflammaging – in the ageing cochlea: a novel target for future presbycusis therapy, Ageing Res. Rev. 40 (2017) 142–148, https://doi.org/10.1016/j.arr.2017.10.002.

[24] S. Emre, T. Karlidag, S. Aydın, I. Kaygusuz, E. Keles, A. Akyigit, S. Yalcin, Can prestin level be a biomarker for determining sensorineural hearing loss? Auris Nasus Larynx 49 (2022) 368–373, https://doi.org/10.1016/j.anl.2021.09.010.

[25] M. Falah, M. Houshmand, M. Najafi, M. Balali, S. Mahmoudian, A. Asghari, H. Emamdjomeh, M. Farhadi, The potential role for use of mitochondrial DNA copy number as predictive biomarker in presbycusis, Therapeut. Clin. Risk Manag. 12 (2016) 1573–1578, https://doi.org/10.2147/TCRM.S117491.

[26] K.O. Tawfik, K. Klepper, J. Saliba, R.A. Friedman, Advances in understanding of presbycusis, J. Neurosci. Res. 98 (2020) 1685–1697, https://doi.org/10.1002/jnr.24426.

[27] C. Verschuur, A. Agyemang-Prempeh, T.A. Newman, Inflammation is associated with a worsening of presbycusis: evidence from the MRC national study of hearing, Int. J. Audiol. 53 (2014) 469–475, https://doi.org/10.3109/14992027.2014.891057.

[28] J.C. Alvarado, V. Fuentes-Santamaría, P. Melgar-Rojas, M.L. Valero, M.C. Gabaldón-Ull, J.M. Miller, J.M. Juiz, Synergistic effects of free radical scavengers and cochlear vasodilators: a new otoprotective strategy for age-related hearing loss, Front. Aging Neurosci. 7 (2015), https://doi.org/10.3389/fnagi.2015.00086.

[29] J.C. Alvarado, V. Fuentes-Santamaría, M.C. Gabaldón-Ull, J.M. Juiz, An oral combination of vitamins A, C, E, and Mg++ improves auditory thresholds in age-related hearing loss, Front. Neurosci. 12 (2018), 10/gd26px.

[30] J.C. Alvarado, V. Fuentes-Santamaría, M.C. Gabaldón-Ull, J.M. Juiz, Age-related hearing loss is accelerated by repeated short-duration loud sound stimulation, Front. Neurosci. 13 (2019) 77, https://doi.org/10.3389/fnins.2019.00077.

[31] E. Tavanai, G. Mohammadkhani, Role of antioxidants in prevention of age-related hearing loss: a review of literature, Eur. Arch. Oto-Rhino-Laryngol. 274 (2017) 1821–1834, 10/f945p2.

[32] R.A. Davies, Audiometry and other hearing tests, in: Handb. Clin. Neurol., Elsevier, 2016, pp. 157–176, https://doi.org/10.1016/B978-0-444-63437-5.00011-X.

[33] F.E. Musiek, J. Shinn, G.D. Chermak, D.-E. Bamiou, Perspectives on the pure-tone audiogram, J. Am. Acad. Audiol. 28 (2017) 655–671, https://doi.org/10.3766/jaaa.16061.

[34] M. Zakaria, The limitations of pure-tone audiometry (as the gold standard test of hearing) that are worthy of consideration, Indian J. Otol. 27 (2021) 1, https://doi.org/10.4103/indianjotol.indianjotol_11_21.

[35] A.C. Carl, M.H. Hohman, J. Cornejo, Audiology pure tone evaluation, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2024. http://www.ncbi.nlm.nih.gov/books/NBK580531/. (Accessed 8 November 2024).

[36] J.C. Alvarado, V. Fuentes-Santamaría, T. Jareño-Flores, J.L. Blanco, J.M. Juiz, Normal variations in the morphology of auditory brainstem response (ABR) waveforms: a study in wistar rats, Neurosci. Res. 73 (2012) 302–311, https://doi.org/10.1016/j.neures.2012.05.001.

[37] E. Domarecka, H. Olze, A.J. Szczepek, Auditory brainstem responses (ABR) of rats during experimentally induced tinnitus: literature review, Brain Sci. 10 (2020) 901, https://doi.org/10.3390/brainsci10120901.

[38] G.W. Overbeck, M.W. Church, Effects of tone burst frequency and intensity on the auditory brainstem response (ABR) from albino and pigmented rats, Hear. Res. 59 (1992) 129–137.

[39] X. Zhou, P.H.-S. Jen, K.L. Seburn, W.N. Frankel, Q.Y. Zheng, Auditory brainstem responses in 10 inbred strains of mice, Brain Res. 1091 (2006) 16–26, https://doi.org/10.1016/j.brainres.2006.01.107.

[40] S. Khullar, R. Babbar, Presbycusis and auditory brainstem responses: a review, Asian Pac. J. Trop. Dis. 1 (2011) 150–157, https://doi.org/10.1016/S2222-1808(11)60056-X.

[41] T.S. Kim, J.W. Chung, Evaluation of age-related hearing loss, Korean J. Audiol. 17 (2013) 50, https://doi.org/10.7874/kja.2013.17.2.50.

[42] K.H. Lee, D.W. Lee, B.C. Kang, The 'R' principles in laboratory animal experiments, Lab. Anim. Res. 36 (2020) 45, https://doi.org/10.1186/s42826-020-00078-6.

[43] J.C. Alvarado, V. Fuentes-Santamaría, M.C. Gabaldón-Ull, T. Jareño-Flores, J.M. Miller, J.M. Juiz, Noise-induced "toughening" effect in Wistar rats: enhanced auditory brainstem responses are related to calretinin and nitric oxide synthase upregulation, Front. Neuroanat. 10 (2016), https://doi.org/10.3389/fnana.2016.00019.

[44] J.C. Alvarado, V. Fuentes-Santamaría, P. Melgar-Rojas, M.C. Gabaldón-Ull, J.J. Cabanes-Sanchis, J.M. Juiz, Oral antioxidant vitamins and magnesium limit noise-induced hearing loss by promoting sensory hair cell survival: role of antioxidant enzymes and apoptosis genes, Antioxidants 9 (2020) 1177, https://doi.org/10.3390/antiox9121177.

[45] V. Fuentes-Santamaría, J.C. Alvarado, J.M. Juiz, Long-term interaction between microglial cells and cochlear nucleus neurons after bilateral cochlear ablation, J. Comp. Neurol. 520 (2012) 2974–2990, https://doi.org/10.1002/cne.23088.

[46] V. Fuentes-Santamaría, J.C. Alvarado, M.C. Gabaldón-Ull, J.M. Juiz, Upregulation of insulin-like growth factor and interleukin 1β occurs in neurons but not in glial cells in the cochlear nucleus following cochlear ablation: upregulation of IGF-1 and IL-1β in Cochlear Nucleus, J. Comp. Neurol. 521 (2013) 3478–3499, https://doi.org/10.1002/cne.23362.

[47] V. Fuentes-Santamaría, J.C. Alvarado, D.F. López-Muñoz, P. Melgar-Rojas, M.C. Gabaldón-Ull, J.M. Juiz, Glia-related mechanisms in the anteroventral cochlear nucleus of the adult rat in response to unilateral conductive hearing loss, Front. Neurosci. 8 (2014), https://doi.org/10.3389/fnins.2014.00319.

[48] P. Melgar-Rojas, J.C. Alvarado, V. Fuentes-Santamaría, M.C. Gabaldón-Ull, J.M. Juiz, Validation of reference genes for RT–qPCR analysis in noise–induced hearing loss: a study in Wistar rat, PLoS One 10 (2015) e0138027, https://doi.org/10.1371/journal.pone.0138027.

[49] X.-J. Yu, X.-X. Xu, S. He, J. He, Change detection by thalamic reticular neurons, Nat. Neurosci. 12 (2009) 1165–1170, https://doi.org/10.1038/nn.2373.

[50] B. Yi, C. Wu, R. Shi, K. Han, H. Sheng, B. Li, L. Mei, X. Wang, Z. Huang, H. Wu, Long-term administration of salicylate-induced changes in BDNF expression and CREB phosphorylation in the auditory cortex of rats, Otol. Neurotol. 39 (2018) e173–e180, https://doi.org/10.1097/MAO.0000000000001717.

[51] V. Fuentes-Santamaría, J.C. Alvarado, S. Mellado, P. Melgar-Rojas, M.C. Gabaldón-Ull, J.J. Cabanes-Sanchis, J.M. Juiz, Age-related inflammation and oxidative stress in the cochlea are exacerbated by long-term, short-duration noise stimulation, Front. Aging Neurosci. 14 (2022) 853320, https://doi.org/10.3389/fnagi.2022.853320.

[52] B. Gourévitch, T. Doisy, M. Avillac, J.-M. Edeline, Follow-up of latency and threshold shifts of auditory brainstem responses after single and interrupted acoustic trauma in Guinea pig, Brain Res. 1304 (2009) 66–79, https://doi.org/10.1016/j.brainres.2009.09.041.

[53] M. Subramaniam, D. Henderson, P. Campo, V. Spongr, The effect of "conditioning" on hearing loss from a high frequency traumatic exposure, Hear. Res. 58 (1992) 57–62.

[54] M.-O. Trowe, H. Maier, M. Schweizer, A. Kispert, Deafness in mice lacking the T-box transcription factor Tbx18 in otic fibrocytes, Development 135 (2008) 1725–1734, https://doi.org/10.1242/dev.014043.

[55] M.W. Church, K.-L.C. Jen, J.I. Anumba, D.A. Jackson, B.R. Adams, J.W. Hotra, Excess omega-3 fatty acid consumption by mothers during pregnancy and lactation caused shorter life span and abnormal ABRs in old adult offspring, Neurotoxicol. Teratol. 32 (2010) 171–181, https://doi.org/10.1016/j.ntt.2009.09.006.

[56] J. Popelar, J. Grecova, N. Rybalko, J. Syka, Comparison of noise-induced changes of auditory brainstem and middle latency response amplitudes in rats, Hear. Res. 245 (2008) 82–91, https://doi.org/10.1016/j.heares.2008.09.002.

[57] V. Fuentes-Santamaría, J.C. Alvarado, P. Melgar-Rojas, M.C. Gabaldón-Ull, J.M. Miller, J.M. Juiz, The role of glia in the peripheral and central auditory system following noise overexposure: contribution of TNF-α and IL-1β to the pathogenesis of hearing loss, Front. Neuroanat. 11 (2017), https://doi.org/10.3389/fnana.2017.00009.
[58] E. Borg, Auditory thresholds in rats of different age and strain. A behavioral and electrophysiological study, Hear, Res. 8 (1982) 101–115.
[59] K.H. Chiappa, K.J. Gladstone, R.R. Young, Brain stem auditory evoked responses: studies of waveform variations in 50 normal human subjects, Arch. Neurol. 36 (1979) 81–87.
[60] D. Konrad-Martin, M.F. Dille, G. McMillan, S. Griest, D. McDermott, S.A. Fausti, D.F. Austin, Age-related changes in the auditory brainstem response, J. Am. Acad. Audiol. 23 (2012) 18–35, https://doi.org/10.3766/jaaa.23.1.3.
[61] A.R. Fetoni, A. Pisani, R. Rolesi, F. Paciello, A. Viziano, A. Moleti, R. Sisto, D. Troiani, G. Paludetti, C. Grassi, Early noise-induced hearing loss accelerates presbycusis altering aging processes in the cochlea, Front. Aging Neurosci. 14 (2022) 803973, https://doi.org/10.3389/fnagi.2022.803973.
[62] I. Ichimiya, M. Suzuki, G. Mogi, Age-related changes in the murine cochlear lateral wall, Hear. Res. 139 (2000) 116–122, https://doi.org/10.1016/S0378-5955(99)00170-7.
[63] S.-H. Sha, A. Kanicki, G. Dootz, A.E. Talaska, K. Halsey, D. Dolan, R. Altschuler, J. Schacht, Age-related auditory pathology in the CBA/J mouse, Hear. Res. 243 (2008) 87–94, https://doi.org/10.1016/j.heares.2008.06.001.
[64] E.M. Keithley, Pathology and mechanisms of cochlear aging, J. Neurosci. Res. 98 (2019) 1674–1684, https://doi.org/10.1002/jnr.24439.
[65] Y. Sergeyenko, K. Lall, M.C. Liberman, S.G. Kujawa, Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline, J. Neurosci. 33 (2013) 13686–13694, https://doi.org/10.1523/JNEUROSCI.1783-13.2013.
[66] M.A. Gratton, B.J. Smyth, B.A. Schulte, D.A. Vincent, Na,K-ATPase activity decreases in the cochlear lateral wall of quiet-aged gerbils, Hear. Res. 83 (1995) 43–50, https://doi.org/10.1016/0378-5955(94)00188-V.
[67] M.A. Gratton, R.A. Schmiedt, B.A. Schulte, Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis, Hear. Res. 102 (1996) 181–190, https://doi.org/10.1016/S0378-5955(96)90017-9.
[68] H. Hibino, K. Higashi-Shingai, A. Fujita, K. Iwai, M. Ishii, Y. Kurachi, Expression of an inwardly rectifying K+ channel, Kir5.1, in specific types of fibrocytes in the cochlear lateral wall suggests its functional importance in the establishment of endocochlear potential, Eur. J. Neurosci. 19 (2004) 76–84, https://doi.org/10.1111/j.1460-9568.2004.03092.x.
[69] J. Popelar, D. Groh, J. Pelánová, B. Canlon, J. Syka, Age-related changes in cochlear and brainstem auditory functions in Fischer 344 rats, Neurobiol. Aging 27 (2006) 490–500, https://doi.org/10.1016/j.neurobiolaging.2005.03.001.
[70] P.M. Backoff, D.M. Caspary, Age-related changes in auditory brainstem responses in Fischer 344 rats: effects of rate and intensity, Hear. Res. 73 (1994) 163–172.
[71] F.A. Boettcher, Presbyacusis and the auditory brainstem response, J. Speech Lang. Hear. Res. 45 (2002) 1249–1261.
[72] T.J. Chen, S.S. Chen, Generator study of brainstem auditory evoked potentials by a radiofrequency lesion method in rats, Exp. Brain Res. 85 (1991) 537–542.
[73] G.V. Simpson, R.T. Knight, S. Brailowsky, O. Prospero-Garcia, D. Scabini, Altered peripheral and brainstem auditory function in aged rats, Brain Res. 348 (1985) 28–35, https://doi.org/10.1016/0006-8993(85)90355-5.
[74] C. Reichmuth, J. Mulsow, J.J. Finneran, D.S. Houser, A.Ya Supin, Measurement and response characteristics of auditory brainstem responses in pinnipeds, Aquat. Mamm. 33 (2007) 132–150, https://doi.org/10.1578/AM.33.1.2007.132.
[75] J. Harrison, J. Buchwald, Auditory brainstem responses in the aged cat, Neurobiol. Aging 3 (1982) 163–171.
[76] E.D. Borre, J.R. Dubno, E.R. Myers, S.D. Emmett, J.M. Pavon, H.W. Francis, O. Ogbuoji, G.D. Sanders Schmidler, Model-projected cost-effectiveness of adult hearing screening in the USA, J. Gen. Intern. Med. 38 (2023) 978–985, https://doi.org/10.1007/s11606-022-07735-7.
[77] A.M. Linssen, L.J.C. Anteunis, M.A. Joore, The cost-effectiveness of different hearing screening strategies for 50- to 70-year-old adults: a Markov model, Value Health 18 (2015) 560–569, https://doi.org/10.1016/j.jval.2015.03.1789.
[78] A.E. Morris, M.E. Lutman, A.J. Cook, D. Turner, An economic evaluation of screening 60- to 70-year-old adults for hearing loss, J. Public Health 35 (2013) 139–146, https://doi.org/10.1093/pubmed/fds058.
[79] A.C. Carl, M.H. Hohman, J. Cornejo, Audiology Pure Tone Evaluation, 2023.
[80] J. Johnson, J. Xu, R. Cox, P. Pendergraft, A comparison of two methods for measuring listening effort as part of an audiologic test battery, Am. J. Audiol. 24 (2015) 419–431, https://doi.org/10.1044/2015_AJA-14-0058.
[81] L.P. Assadouro, C.M. Silva, C.D. Reis, C. Nazaré, S. Paulo, M. Serrano, Listening effort, an overview of app validation and testing by the audiology 4 all Project, Int. Tinnitus J. 27 (2023), https://doi.org/10.5935/0946-5448.20230016.
[82] S. Alhanbali, P. Dawes, R.E. Millman, K.J. Munro, Measures of listening effort are multidimensional, Ear Hear. 40 (2019) 1084–1097, https://doi.org/10.1097/AUD.0000000000000697.
[83] B. Jalaei, A. Valadbeigi, R. Panahi, M.H. Nahrani, H.N. Arefi, M. Zia, N. Ranjbar, Central auditory processing tests as diagnostic tools for the early identification of elderly individuals with mild cognitive impairment, J. Audiol. Otol. 23 (2019) 83–88, https://doi.org/10.7874/jao.2018.00283.
[84] J. Joseph, C. Niemczak, J. Lichtenstein, A. Kobrina, A. Magohe, S. Leigh, C. Ealer, A. Fellows, C. Reike, E. Massawe, J. Gui, J.C. Buckey, Central auditory test performance predicts future neurocognitive function in children living with and without HIV, Sci. Rep. 14 (2024) 2712, https://doi.org/10.1038/s41598-024-52380-1.