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Research article

Senescence-accelerated mouse prone 8 (SAMP8) as a model of age-related hearing loss

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HIGHLIGHTS

- Senescence degeneration of auditory system in SAMP8 strain.
- Increase of ABR thresholds from 37 days old in SAMP8 mouse model.
- Decrease of DPOAE amplitude in SAMP8 mice from 37 days old at mid frequencies.

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ABSTRACT

Hearing loss is the most common form of sensory impairment in humans, affecting 5.3% worldwide population. In industrial countries, age-related hearing loss is a major health problem affecting one-third of individuals over 65 years old. However, the physiological and molecular changes involved in this senescence process remain unclear. In this study, we determined the influence of age on auditory brainstem response (ABR) and the distortion product otoacoustic emissions (DPOAE) in the premature senescence mouse model SAMP8 for five months. We showed a progressive increase of ABR thresholds and a decrease of distortion product amplitude from 37 days old in SAMP8 compared to CBA mice. The data we show here provide new knowledge in functional auditory changes during the senescence process and open up new opportunities for the development of new drugs involved in age-related hearing loss treatment.

1. Introduction

Age-related hearing loss (ARHL) is the most common sensory disorder in the elderly population [1,2]. This disorder is characterized by symmetric sensorineural hearing loss that starts at high frequencies with a prevalence of 35% of individuals over 65 years [3]. ARHL impact the quality of life and the more severe forms affect communication leading to social isolation, depression and reduced physical and psychological wellbeing. Although hearing loss has been considered to be part of a natural aging process, heritability studies suggest that the source of variability is both genetic and environmental. Interestingly, studies have established associations between ARHL and genes linked to oxidative stress detoxification, such as arylamine *N*-acetyltransferase 2 and glutathione *S*-transferase [4–6]. However, the molecular mechanisms underlying ARHL are unknown, and currently there is no treatment for this disorder.

The senescence-accelerated prone strain 8 (SAMP8) mouse model is a strain derived from AKR/J mice, selected for a phenotype toward either accelerated senescence with a mean life span of 9.7 months [7]. SAMP8 mice have been identified as suitable for use as models in gerontological research [8]. This strain has been widely used in aging research to study phenotypes such as immune dysfunction, osteoporosis, and brain atrophy [7,8]. It has been reported that SAMP8 strain displays sequential degeneration of outer hair cells (OHCs), spiral ganglion neurons (SGNs), stria vascularis and ultimately inner hair cells (IHCs) which mimic human ARHL [9,10].

Moreover, a recent study demonstrated that the molecular mechanisms associated with premature SAMP8 senescence involve oxidative stress, altered levels of anti-oxidant enzymes and decreased activity of Complexes I, II and IV which in turn lead to chronic inflammation and triggering of apoptotic cell death pathways [10]. Together, these data indicate that SAMP8 model could

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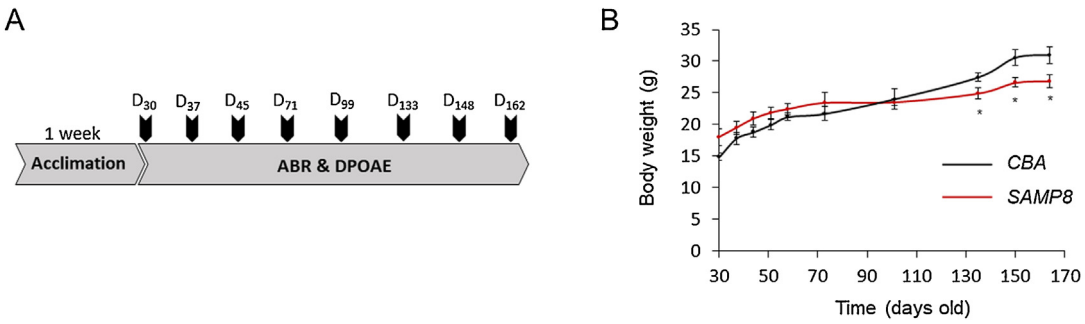

Fig. 1. A. Schema of the experimental study. B. Body weight evolution of SAMP8 and CBA mice during the study. Data are shown as mean ± SEM. Significance was set at *p < 0.05. *n* = 8 mice by group.

play an essential role in the development of effective ARHL treatments.

To characterize this ARHL model we measured the auditory brainstem response (ABR) and the distortion product otoacoustic emissions (DPOAEs), two auditory parameters, in SAMP8 mice for five months, compared to CBA/J RJ mice as control.

2. Material and methods

2.1. Animals

SAMP8/TaHsd and CBA/J RJ female mice (weight 17–20 g, 3 weeks old), were obtained from Envigo and Janvier-Labs respectively and maintained under specific pathogen-free conditions. Before starting the study, we allowed the mice to acclimatize for 7 days. Mice were kept in the animal facility in clear macrolon cages at 22 ± 2 °C, subjected to standard light cycles and with standards diet and water ad libitum. All animal experiments were conducted in accordance with the French Institutional and National Regulation A34-169-002.

2.2. Auditory brainstem response (ABR)

For ABR studies, mice were anesthetized using a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and the tragus was removed using sterile scissors in order to open the external auditory canal. Then, animals were placed in an acoustic chamber to completely isolate them from exterior noise and on a thermostatically regulated heating pad to maintain the temperature at 37 °C. Animals were placed by group of eight and earphones (Philips SHE8500) were placed in the left ear of each mouse. An active electrode is placed in the vertex of the skull, a reference electrode under the skin of the mastoid bone and a ground electrode is placed in the neck skin. The electrophysiological signals were amplified with a gain of 10,000 and filtered between 100 and 1000 Hz (amplifiers AM systems model 1700) and then converted into digital signal and stored on a computer for later analysis. The stimuli were consisted of bips with center frequency of 2, 4, 8, 16 and 32 kHz, to cover the mice auditory frequency range. These series of bips were presented at various sound levels ranging from 90 to 10 dB with 10 dB steps. Evoked potentials were extracted by the signal averaging technique for each noise level.

2.3. Distortion product otoacoustic emission (DPOAE)

For DPOAE measures, mice were anesthetized using ketamine/xylazine mixture and a probe was inserted into the external left ear canal. The measurement system used is Otophylab, which has the capacity to send a succession of sounds in f1 and f2 frequencies. The primary tone F_2 was set at 1, 1.5, 2, 3, 4, 5, 8, 12, 16, 24 and 32 kHz. The frequency ratio F_2/F_1 was set at 1.2. Both F_2 and F_1 were given an intensity of 58 dB SPL. At all frequencies (F_2), the input of the DPOAE system received, digitized and evaluated the output of the microphone using the system software.

2.4. Statistical analysis

Descriptive statistics by groups and frequencies are provided with MEDIAN ± MAD (median ± Median Absolute Deviation) for ABR measures and with MEAN ± SEM (arithmetic mean ± Standard Error of the Mean) for animal body weight and DPOAE measures. A 2-way analysis of the covariance on ranks with repeated measurements on frequencies with animal's baseline value as covariate was performed. A spherical correlation structure between frequency was used. Post-hoc analysis to compare SAMP8 versus CBA group at each frequency was performed with the Dunnett p-value adjustment for multiplicity correction.

3. Results

3.1. Decrease of SAMP8 body weight at advanced stage of senescence process

In this study, the animal weight and auditory functions have been assessed in SAMP8/TaHsd and CBA/J RJ mice from 30 to 162 days old (Fig. 1A) in order to determine the influence of the age in body weight and peripheral and central auditory pathway impairment. We observed a significant weight reduction of SAMP8 compared to CBA mice at the advanced stages of senescence process (Fig. 1B). Whereas the body mass of CBA remained stable around 30 ± 1.3 g at 150 and 164 days old, the body weight of SAMP8 reached a plateau of 26.7 ± 0.9 g at the same age. These body weight changes can be explained by the decrease of muscle mass due to the advanced stage of the senescence process as previously described Guo and collaborators [11].

3.2. Increase of ABR thresholds in SAMP8 mice

ABRs are electric potentials recorded from scalp electrodes, and the first ABR wave represents the summed activity of the auditory nerve fibers contacting the IHCs. ABR thresholds were similar between CBA and SAMP8 mice at 30 days old (Fig. 2A) but a significant increase of thresholds at 8 and 32 kHz were observed from 37 days old (Fig. 2B) in SAMP8 mice. At 45 days old, this increase of ABR thresholds were also observed at 2 and 4 kHz (Fig. 2C) and these electrophysiological differences became more significant at all analyzed frequency from 71 days old with a progressive increase until the end of the study (162 days old) (Fig. 2D–H). This progressive auditory impairment could be explained by the peripheral and

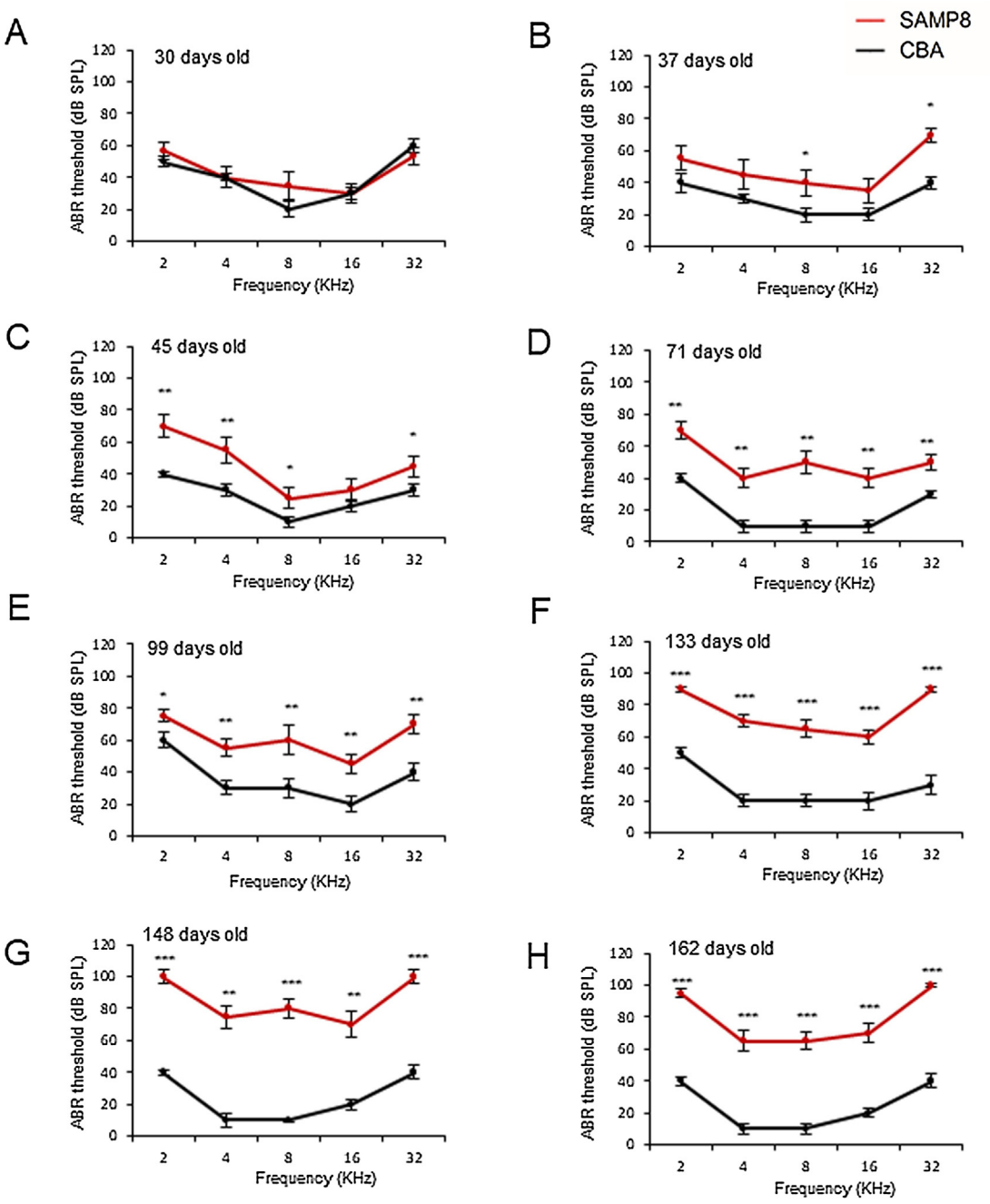

Fig. 2. Auditory Brainstem Response thresholds of SAMP8 (red line) and CBA mice (black) at 2, 4, 8, 16 and 32KHz at the indicated age. The age of the mice is indicated on each graph (days old). Data are shown as median ± MAD. Significance was set at *p < 0.05, ** p < 0.01, or ***p < 0.001. *n* = 8 mice by group.

central nerve degeneration [9] as well as the loss of sensory hair cells [10] previously published.

Some small ABR threshold improvements were observed in some frequencies during the time course experiment, for example at 32 kHz in SAMP8 mice at 45 days old (Fig. 2C) compared to 37 days old (Fig. 2B). Because not statistical differences were observed between these points, the threshold level changes can be explained by the natural biological variability of the animals along the time point measures. Moreover, a large number of studies demonstrated that anesthesia can weakly modify ABR thresholds [12] suggesting that these small level changes could be also explained by a deep anesthesia state during the electrophysiological measure.

3.3. Decrease of DPOAE amplitude in SAMP8 mice

On the other hand, DPOAEs are acoustic signals created and amplified by the cochlear epithelium offering an index of cochlear function and are linked to outer hair cell health (OHCs) [13] which amplify sound-evoked cochlear vibration. They do not depend on IHCs or auditory nerve fibers. It has long been established that DPOAE decrease in amplitude with increasing hearing loss [14] but there is also evidence that aging, independent of hearing loss, reduces otoacoustic emissions amplitude [15]. Using this electroacoustic technique, we showed that DPOAE amplitude of SAMP8 and CBA was similar at 30 days old (Fig. 3A) but a significant decrease of amplitude was observed from 37 days old at mid frequencies (between 12 and 24 KHz) (Fig. 3B) and this DPOAE amplitude

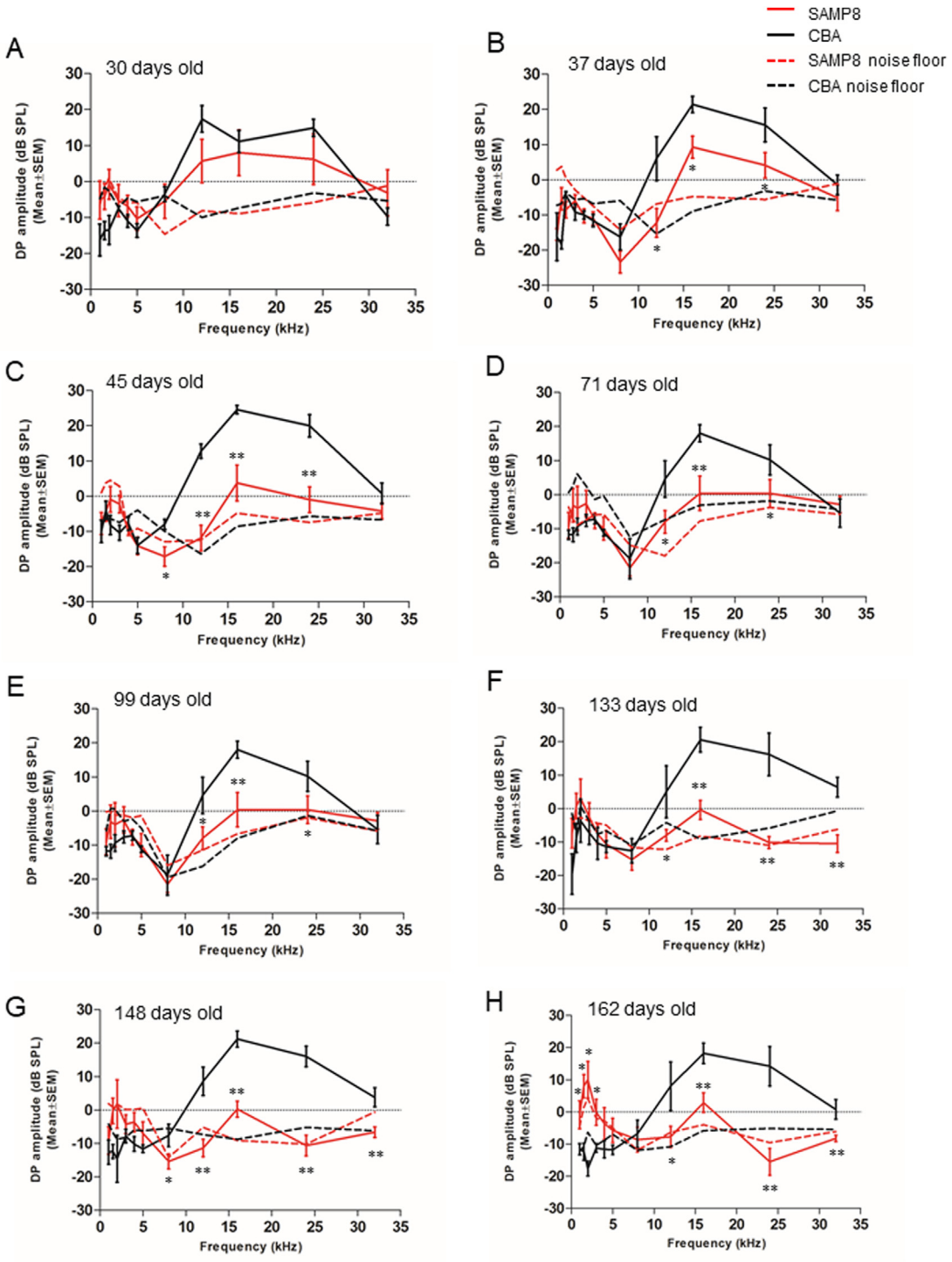

Fig. 3. DPOAE amplitude level of SAMP8 (red line) and CBA mice (black line) at 1, 4, 6, 8, 10, 12, 16, 20, 25 and 32 kHz and an intensity of 58 dB SPL. The age of the mice is indicated on each graph (days old). Discontinuous lines represent the background noise floor. Data are shown as mean ± SEM. Significance was set at $^{*}p < 0.05$ and $^{**}p < 0.01$. $n = 8$ mice by group.

decreased progressively along the senescence process (Fig. 3B–H). Moreover, the DPOAE amplitude became significantly reduced at the high frequency (33 KHz) from 133 days old (Fig. 3F–H), showing the advanced stage of the senescence process.

Interestingly, a significant increase of DPOAE level was observed at the low frequencies (from 1 to 3 kHz) at 162 days old in SAMP8 mice compared to CBA mice. However, when we analyzed the background noise at this age, we observed a strongly increase of noise floor in SAMP8 compared to CBA and the distortion product response did not exceed this background noise. As Menardo and collaborators published, [10] SAMP8 model expresses cochlear oxidative stress leading to progressive stria vascularis degeneration and strong tissue inflammation. Because DPOAE background noise depends on the cochlear tissue states, the increase of noise floor could be explained by the inflammation observed at the advanced senescence stages. So, we hypothesized that the increase of DPOAE level should not be due to an increase of OHC cell number or activity but an increase of noise floor due to aging physiological changes.

4. Discussion

Here we study the age-related hearing loss using SAMP8 mice and ABR and DPOAE measures to follow functional changes from the peripheral to the central auditory system during the accelerated senescence. We observed a hearing impairment in the mid frequencies from 37 days old determined by ABR thresholds measure. At the same mouse age, DPOAE amplitude significantly decreased also in the mid frequencies suggesting that the ABR threshold increase could be due to a decrease of OHC number or functionality. Because ABR and DPOAE performance impairments followed the same time course during the senescence process of SAMP8 mice, the increase of ABR thresholds could be due by the reduction of the cochlear hair cell number. Moreover, Menardo and collaborators published that SAMP8 mice present an early and progressive degeneration of OHC using immunolabeling and electron microscopy [10]. So, these results corroborate our functional study data suggesting that the decrease of ABR and DPOAE performance were due to a decrease of the OHC number in SAMP8 cochlea.

Senescent-accelerated resistant 1 mouse strain (SAMR1) is usually used as SAMP8 control as it presents normal senescence. However, some studies demonstrated that SAMR1 express ARHL expressed as elevated thresholds and decrease of spiral ganglion neurons size [16] as some pathobiological phenotypes as non-thymic lymphoma and histiocytic sarcoma [17]. For these reasons, we decided to use the CBA/J RJ mice as a control, a strain genetically and physiologically well characterized with a normal senescence process.

Several mouse models have been proposed to mimic the different forms of human ARHL. Particularly, the C57BL/6J mouse strain presents auditory functional deficits, accompanied by the loss of cochlear hair cells and neurons. The central neural changes resulted in a widespread tonotopic reorganization that ultimately increases the low-frequency representation to invade much of the auditory cortex and the inferior colliculus [18]. However, this mouse strain displays the classic pattern of hearing loss at 12–15 months of age [19] so C57BJ/6J cannot be used routinely for ARHL studies due to the time-consuming model. For this reason, and because SAMP8 mice mimic closely other human age-related diseases as dementia and diabetes in the early age stages, this strain is presented as the fastest and most robust model for ARHL drug candidates screening.

It is well known that cochleae are vulnerable to reactive oxidative species (ROS) because of the high metabolic demands of their mechanosensory hair cells in response to sound stimulation. Normally, ROS produced by hair cell are scavenged by endogenous antioxidant mechanisms. However, during senescence ROS concentration increase significantly leading to genetic and cellular alterations which cause cellular dysfunctions such as lipid peroxidation, mitochondrial DNA mutation and enzyme inactivation leading to permanent cochlear degeneration [20]

Importantly, oxidative damage has been universally observed in the SAMP8. Nomura and collaborators were the first to find higher amounts of malondialdehyde and lower levels of superoxidase dismutase in SAMP8 compared to control [21]. Moreover, SAMP8 cochlear damages have also been associated to increase of ROS and alterations of antioxidant enzymes that lead to chronic inflammation and cochlear hair cell apoptosis [10]. In this way, antioxidants and free radical scavengers may serve as effective therapeutic agents to block the activation of death mechanisms induced by age-related ROS overproduction [22]. Interestingly, clinical and military trials have been carried out for temporary threshold shift, in which administration of antioxidant supplements, before moderate noise exposure showed beneficial auditory effects [23]. So, targeting members of antioxidant pathways, including the enzymes that are involved in the breakdown of superoxide anions and hydrogen peroxidase, could be feasible options for the treatment of several types of hearing loss, including ARHL [22].

5. Conclusion

In summary, we showed that ABR thresholds were increased from 37 days old in SAMP8 mice compared to CBA and these differences became more significant along the accelerated senescence process. In parallel, decrease of DPOAE amplitude was also observed in SAMP8 mice from 37 days old at mid frequencies and this amplitude became strongly reduced at the high frequency at 133 days old, showing the advanced stage of the senescence process.

Because the characterization of ARHL models is the first step for the research of compounds involved in hearing recovery, the data we present here could allow the development of drug effect studies on the progression of ARHL in short timeframe.

Author's contributions

AM performed ABR and DPOAE measures, PLC set up ABR recording system, SCP designed the study and participated at the manuscript redaction and SGG analyzed and interpreted data and wrote the manuscript. All authors approved the final version of the manuscript and agree to be held accountable for the work therein.

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Competing interests

Authors declare no competing interests.

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