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Research Article

Physiological Evidence for Delayed Age-related Hearing Loss in Two Long-lived Rodent Species (*Peromyscus leucopus* and *P. californicus*)

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ABSTRACT

Deer mice (genus *Peromyscus*) are an emerging model for aging studies due to their longevity relative to rodents of similar size. Although *Peromyscus* species are well-represented in genetic, developmental, and behavioral studies, relatively few studies have investigated auditory sensitivity in this genus. Given the potential utility of *Peromyscus* for investigations of age-related changes to auditory function, we recorded auditory brainstem responses (ABRs) in two *Peromyscus* species, *P. californicus*, and *P. leucopus*, across the lifespan. We compared hearing sensitivity and ABR wave metrics measured in these species with measurements from *Mus musculus* (CBA/CaJ strain) to assess age-related effects on hearing across species. Recordings in young animals showed that all species had similar hearing ranges and thresholds with peak sensitivity ranging from 8 to 16 kHz; however, *P. californicus* and *P. leucopus* were more sensitive to frequencies below 8 kHz. Although *M. musculus* showed significant threshold shifts across a broad range of frequencies beginning at middle age and worsening among old individuals, older *Peromyscus* mice retained good sensitivity to sound across their lifespan. Middle-aged *P. leucopus* had comparable thresholds to young for frequencies below 24 kHz. *P. leucopus* also had notably large ABRs that were robust to age-related amplitude reductions, although response latencies increased with age. Old *P. californicus* were less sensitive to mid-range tones (8–16 kHz)

than young individuals; however, there were no significant age-effects on ABR amplitudes or latencies in this species. These results indicate that longevity in *Peromyscus* mice may be correlated with delayed aging of the auditory system and highlight these species as promising candidates for longitudinal hearing research.

Keywords: Auditory brainstem response, Aging, Alternative model, Comparative study, Deer mouse, Presbycusis

INTRODUCTION

Hearing in mammals gradually deteriorates with age, leading to compromised abilities to respond to environmental stimuli, detect prey or predators, and interact with conspecifics. In humans, age-related hearing loss (ARHL) is the most common sensory impairment (Collins 1997; Goman and Lin 2016), and, for half of people over 70, these impairments are severe enough to limit communication (Agrawal et al. 2008; Yamasoba et al. 2013; Bowl and Dawson 2019). The implications of ARHL in humans are profound as diminishing abilities to communicate contribute to social isolation, cognitive decline, and decreased quality of life (Lin et al. 2011; Loughrey et al. 2018; Rutherford et al. 2018). Regardless of the prevalence and negative health outcomes associated with ARHL, our understanding of the biological mechanisms that influence this condition remains incomplete.

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Most studies of the aging mammalian auditory system leverage the house mouse (*Mus musculus*) as a model, owing to its genetic tractability and ease of care in laboratory conditions. However, the lack of genetic diversity in inbred laboratory strains creates challenges to adequately recapitulate the full range of human disease phenotypes (Voelkl et al. 2020). Further, *M. musculus* is a short-lived species that may not manifest aging patterns comparable to long-lived mammals such as humans (Dammann 2017). *M. musculus* has been additionally criticized as a model for hearing research due to its insensitivity to low frequencies, increased susceptibility to noise, and limited within-strain audiometric variation. The development of alternative rodent models of ARHL has received less attention in auditory neuroscience with the exception of the Mongolian gerbil (*Meriones unguiculatus*), which is valued for its sensitivity to low frequencies in the range relevant for human speech perception (Ohlemiller and Echteler 1990; Heeringa et al. 2020). While these species have been indispensable for understanding basic mechanisms of hearing and deafness, it is important to consider comparative approaches incorporating additional species, especially those that reflect natural audiometric variability and susceptibility to hearing loss.

Deer mice (genus *Peromyscus*) are among the most abundant mammals in North America and are more closely related to voles and hamsters (family Cricetidae) than to mice of the family Muridae. *Peromyscus* species have been studied extensively with regard to their diverse behaviors, reproductive strategies, and physiological adaptations (reviewed in Bedford and Hoekstra 2015). Recently, *Peromyscus* mice have emerged as a valuable model for aging and age-related pathologies due to their substantial longevity relative to comparably sized mammals. Captive *Peromyscus leucopus* demonstrate average lifespans nearly twice that of *M. musculus* in laboratory colonies, with maximal lifespan potentials reaching up to 8 years (Sacher and Hart 1978; Burger and Gochfeld 1992; Guo et al. 1993). Relative to *Mus*, *Peromyscus* mice show reduced production of reactive oxygen species and increased resistance to oxidative stress, leading to delayed accumulation of oxidative damage to DNA over their lifetimes (Sohal et al. 1993; Csiszar et al. 2007; Ungvari et al. 2008; Labinskyy et al. 2009; Shi et al. 2013) that, among other protective effects, may delay cochlear aging (Ohlemiller and Frisina 2008). *Peromyscus* mice therefore may be considered "successfully" aging species—those that demonstrate delayed or reduced age-related phenotypic changes.

Although relatively few studies have investigated hearing in *Peromyscus*, early work suggested that *Peromyscus* species may be more sensitive to low frequencies down to 1 kHz than *Mus* (Ralls 1967). Additionally, *Peromyscus* vocalizations have been well-studied in both wild and laboratory contexts (e.g., Kalcounis-Rueppell et al. 2006, 2010; Briggs and Kalcounis-Rueppell 2011; Miller and

Engstrom 2012; Kobrina et al. 2021a, b), which should facilitate the development of behavioral assays that replicate naturalistic scenarios for investigating context- and state-dependent auditory behaviors. For example, lab-bred *P. californicus* spontaneously emit ultrasonic vocalizations (USV) similar in motif to those produced by wild individuals (Kalcounis-Rueppell et al. 2010). In the wild, male and female *P. californicus* produce stereotyped USV motifs under certain behavioral contexts, such as during affiliative or aversive encounters (Briggs and Kalcounis-Rueppell 2011), suggesting that responses to USVs in this species could be a tractable auditory behavior in the lab. *Peromyscus* mice represent an additional model that may complement auditory research in other rodents and present the opportunity to investigate the aging auditory system in a long-lived, successfully aging species.

Here we used auditory brainstem response (ABR) measurements to evaluate hearing sensitivity in two *Peromyscus* species (*P. californicus* and *P. leucopus*) and in a laboratory-bred strain of *M. musculus* (CBA/CaJ) that represents a normal aging auditory phenotype (Kobrina and Dent 2016, 2020; Vicencio-Jimenez et al. 2021). We tested hearing in these species at three time points representing different stages of their average maximum lifespan to compare age-related changes to hearing among species.

METHODS

Animals

We incorporated male and female *Peromyscus leucopus* ($n = 35$; 21 females, 14 males), *P. californicus* ($n = 12$; 6 females, 6 males), and *Mus musculus* (CBA/CaJ, $n = 36$; 24 females, 12 males) mice ranging from postnatal day 30 to approximately 31 months old. Although the CBA/CaJ strain used in the present study is not a widely used model for rodent studies that more commonly leverage the C57BL/6 J inbred strain, CBA/CaJ mice do not exhibit early onset of ARHL as has been observed in strains derived from the C57 background (Frisina and Walton 2001). Animals were divided into three age groups (young, middle-aged, and old) that were calculated based on percent median lifespan for each species (see Table 1). Some animals were represented across multiple age groups due to repeated testing, including 4 *P. leucopus*, 3 *P. californicus*, and 6 *M. musculus* that were re-tested as young, middle-aged, and/or old individuals. Mice were bred in the laboratory using individuals purchased from the *Peromyscus* Genetic Stock Center at the University of South Carolina (*P. leucopus* and *P. californicus*) and the Jackson Laboratory (CBA/CaJ, strain #000654). Animals were housed in a low-noise (1/3 octave band noise levels less than 30–40 dB SPL), low-traffic vivarium to reduce extraneous noise exposure (Wu et al. 2020). The room

TABLE 1

Age groups and sample sizes for each species

Species	Age group	Age range* (months)	Sample size (females, males)
Mus musculus	Young	2–6	13 (9, 4)
	Middle-aged	7–10	12 (7, 5)[1]
	Old	>12	16 (13, 3)[1]
Peromyscus califor-nicus	Young	3–6	8 (3, 5)
	Middle-aged	10–15	0[2]
	Old	>18	7 (5, 2)[1]
Peromyscus leuco-pus	Young	4–8	11 (7, 4)
	Middle-aged	13–19	21 (14, 7)[1]
	Old	>24	7 (2, 5)

*Age ranges calculated as percent total lifespan based on an approximate median life expectancy of 24.2 months in *M. musculus* (averaged across males and females of 4 strains previously used in hearing loss studies: CBA/J, C57BL/6 J, FVB/NJ, and DBA/2 J, Yuan et al. 2009), 36.7 months in *P. californicus*, and 49.2 months in *P. leucopus* (Sacher and Hart 1978). [1]Some individuals are included in multiple age groups due to repeated testing. [2]We were unable to collect data for middle-aged *P. californicus* due to COVID-related restrictions during the critical time-points

was maintained on a 12:12 h automated light:dark cycle and had a window to provide natural light. Animals had access to ad libitum food and water and were housed in static filter top cages with corncob bedding and nesting material for enrichment that were changed weekly. *Peromyscus* mice also had shredded paper bedding per the recommendations of the *Peromyscus* Genetic Stock Center. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Auditory Brainstem Response Recordings

We recorded ABRs in young adult mice using procedures described in our previous publications (Lauer 2017; Schrode et al. 2018; Kim et al. 2022). Mice were anesthetized with an intraperitoneal injection of 100 mg/kg ketamine and 20 mg/kg xylazine and placed on a heating pad to maintain a temperature of 37 °C. Subdermal needle electrodes were placed at the vertex and the mastoid/ bulla for differential recording, and a ground electrode was inserted in the hind leg.

Free-field sound stimuli were generated, and responses were recorded inside an IAC anechoic recording chamber using Tucker-Davis Technologies (TDT) System III hardware (TDT RZ6 and RX6 Multi I/O processor),

custom built MATLAB software, and SigGen/BioSig software (TDT, Alachua, FL). Acoustic stimuli were presented using a TDT magnetic speaker (model MF1) or a Fostex dome tweeter speaker (model FT28D). Stimuli consisted of clicks (0.1-ms square wave pulses) and tones (5-ms, 0.5 ms rise/fall times) at frequencies of 1, 2, 4, 8, 12, 16, 24, and 32 kHz presented at a rate of 21/s and with alternating polarity to reduce stimulus artifacts. We calibrated stimuli using a 1/4 inch free-field microphone (Brüel and Kjær, type 4939 or PCB, model 426BO3) placed at the position of the animal's ear during ABR recording experiments. We presented click and tone stimuli at levels ranging from 10 to 90 dB re 20 µPa in 5–10 dB decrements until a threshold was reached. Auditory evoked potentials were recorded with a 12 kHz sampling rate. Responses were averaged across 512 stimulus presentations, amplified using an RA4PA Medusa pre-amplifier (TDT, Alachua, FL) or an ISO-80 Differential Amplifier (World Precision Instruments, Sarasota, FL), and filtered using 300 Hz high-pass and 3000 Hz low-pass Butterworth filter.

We defined detection thresholds as the intermediate sound level between the lowest level of stimulation that evoked a response discriminable from noise and the level at which no response was observed. For cases where no thresholds were obtained for stimuli presented at the highest intensities, a threshold value of 95 dB re 20 µPa was used for statistical analyses. Thresholds were determined by visual inspection by three independent experienced observers and using an objective autocorrelation algorithm following methods described in Suthakar and Liberman (2019). We compared click-evoked thresholds determined by each method using a Wilcoxon matched-pairs test and found no statistical differences ($p = 0.7734$); however, tone-evoked ABRs in *Peromyscus* were considerably noisier compared to *Mus*, and the objective method was less effective at detecting ABR waves in these species relative to visual verification by experienced observers. We therefore used the visually determined thresholds for subsequent analyses of ABR data recorded in all species. We measured amplitudes and latencies of ABR waves I–IV evoked by clicks, 8, and 24 kHz stimuli presented at 90 dB re 20 µPa using a custom program, followed by visual verification of the automatically detected peaks and troughs. Amplitudes were measured as the maximum peak-to-trough voltage of each ABR wave, and latencies were measured as the timing of the maximum voltage peak of each wave relative to the timing of stimulus onset.

Data Analysis

We compared ABR thresholds across species using linear mixed-effects models (LMMs) with thresholds derived from young individuals as the response variable, frequency, species, and sex as fixed effects and individual as a random effect. LMMs provide an advantage over standard

analysis of variance procedures because they are robust to the effects of missing data and unbalanced datasets. We evaluated within-species age effects on sensitivity thresholds using LMMs incorporating frequency, sex, and age group as fixed effects and individual as a random effect. Finally, we analyzed within-species age effects on the amplitudes and latencies of ABR waves I–IV incorporating sex and age group as fixed effects. ABR waves for which peaks could not be reliably discriminated from the noise floor were excluded from statistical analyses. For all analyses, we used Akaike's information criterion adjusted for small samples (AICc) to select the best-fit model for our data. This model selection procedure uses maximum likelihood estimation to determine the model that best describes the relationship among the factors tested (Burnham and Anderson 2002). We then evaluated significant differences using type III tests of fixed effects followed by post hoc pairwise comparisons with a Bonferroni correction for multiple comparisons. All statistical analyses were performed in R version 4.0.3 (R Core Team 2020) using the packages MuMIn (Barton 2020), lme4 (Bates et al. 2015), and emmeans (Lenth 2022).

RESULTS

Hearing Sensitivity Across Species

All three species showed best sensitivity (i.e., lowest detection thresholds) to sounds between 8 and 16 kHz (Fig, 1A). We found significant main effects of frequency (LMM: $F_{(8,203.70)} = 181.86$, $p < 2.20E\text{-}16$), species (LMM: $F_{(2,25.36)} = 3.940$, $p = 0.032$), and the interaction of species and frequency (LMM: $F_{(16,203.72)} = 8.334$, $p = 2.65E\text{-}15$). Post hoc comparisons revealed significant interspecific differences in thresholds for all frequencies except 1, 12, and 32 kHz. *P. leucopus* had significantly lower thresholds at 2 kHz than *M. musculus* ($p = 0.036$). For 4 kHz tones, *M. musculus* had higher thresholds than both *P. leucopus* ($p < 0.0001$) and *P. californicus* ($p = 0.0001$). Similarly, *M. musculus* had higher thresholds to 8 kHz tones relative to both *P. leucopus* ($p < 0.0001$) and *P. californicus* ($p < 0.0001$). *P. leucopus* had lower thresholds to 16 kHz relative to both *M. musculus* ($p = 0.014$) and *P. californicus* ($p = 0.0004$). *P. californicus* had higher thresholds than *M. musculus* at 24 kHz ($p = 0.013$). The interaction of species
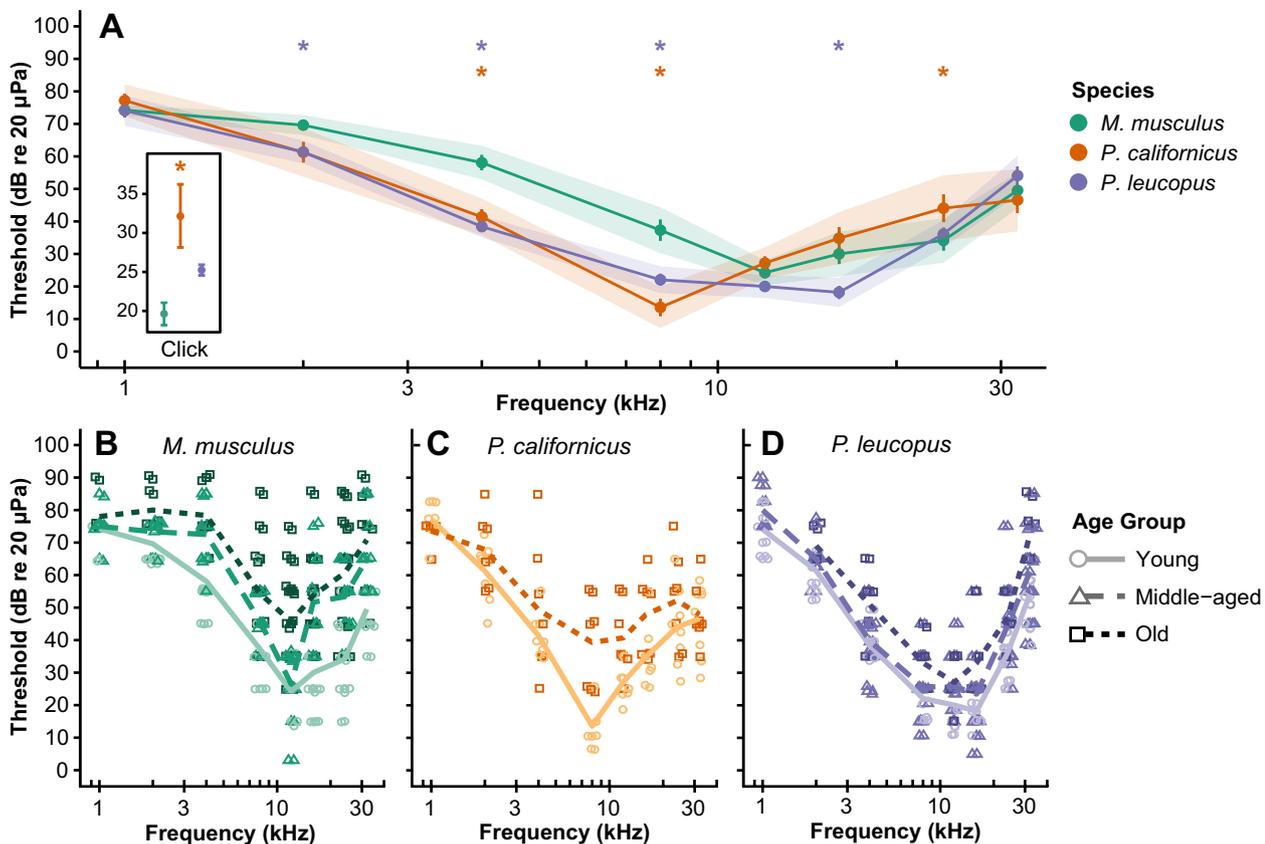
Fig. 1 Comparison of hearing sensitivity among species and across age groups. **A** Audiograms for young *Mus musculus* (CBA/CaJ, $n = 13$), *Peromyscus californicus* ($n = 8$), and *P. leucopus* ($n = 11$). Mean click response thresholds (in dB re 20 µPa) are shown in the inset panel to **A**. Error bars represent standard errors and shaded ribbons indicate 95 % confidence intervals of the mean. Asterisks indicate significant differences in mean thresholds measured in *Peromyscus* species compared to mean thresholds measured in *M. musculus* ($p < 0.05$). Age-group comparisons of thresholds in **B** *M. musculus* (young, $n = 13$; middle-aged, $n = 12$; and old, $n = 16$), **C** *P. californicus* (young, $n = 8$, and old, $n = 7$), and **D** *P. leucopus* (young, $n = 11$; middle-aged, $n = 21$; and old, $n = 7$).

and sex was marginally significant in the original model ($F_{(2,25.36)} = 3.798$, $p = 0.0461$); however, post hoc testing did not return any significant intraspecific variation among sexes for any of the frequencies tested.

Age-related Changes to Hearing Sensitivity

Both middle-aged and old *M. musculus* demonstrated high individual variability in ABR thresholds, particularly in response to clicks and 8–24 kHz tones (Fig. 1B). We observed significant main effects of frequency (LMM: $F_{(8,235.72)} = 115.66$, $p < 2.20E\text{-}16$), age group (LMM: $F_{(2,32.22)} = 21.992$, $p = 9.48E\text{-}7$), and the interaction of age group and frequency (LMM: $F_{(16,235.11)} = 4.182$, $p = 4.17E\text{-}7$). Middle-aged *M. musculus* showed significant increases to mean thresholds recorded in response 4 kHz ($p = 0.0007$), 16 kHz ($p < 0.0001$), 24 kHz ($p < 0.0001$), and 32 kHz tones ($p = 0.001$) relative to young individuals (Fig. 2). Old individuals had elevated thresholds in response to clicks ($p = 0.0001$) and to frequencies from 4 to 32 kHz relative to young individuals (all $p \le 0.0001$, see Supplementary Table S6 for results of all pairwise comparisons).

For *P. californicus*, we found significant main effects of frequency (LMM: $F_{(8,91.27)} = 37.87$, $p < 2.20E\text{-}16$) and the interaction of age group and frequency (LMM: $F_{(8,91.27)} = 3.053$, $p = 0.004$). We observed no significant differences in detection thresholds recorded in young and old individuals to clicks or tones from 1 to 4 kHz and 24 to 32 kHz (Supplementary Table S9). Thresholds recorded from old *P. californicus* individuals were significantly higher than those from young individuals for 8 kHz ($p = 0.0002$), 12 kHz ($p = 0.037$), and 16 kHz ($p = 0.032$) tones (Fig. 2). The mean ABR-derived audiogram for old

P. californicus individuals was shallower than the young audiogram (Fig. 1C), indicating a potential frequency-specific decline in hearing with age. However, thresholds measured from old *P. californicus* individuals were highly variable, ranging from 25 to 55 dB re 20 µPa in response to 8 kHz tones and from 35 to 85 dB in response to 4 kHz tones.

ABR thresholds recorded from *P. leucopus* were significantly influenced by both frequency (LMM: $F_{(8,244.33)} = 175.77$, $p < 2.20E\text{-}16$) and age group (LMM: $F_{(2,32.81)} = 6.798$, $p = 0.0034$). There were no significant differences in *P. leucopus* detection thresholds across age groups for click, 2 kHz, 8 kHz, and 12 kHz responses (Fig. 2). Middle-aged animals had elevated thresholds relative to young individuals for 24 kHz ($p = 0.004$) and 32 kHz ($p = 0.021$). Thresholds recorded from old individuals were significantly higher than those recorded from young animals at 4 kHz ($p = 0.0089$), 16 kHz ($p = 0.0078$), 24 kHz ($p = 0.0469$), and 32 kHz ($p = 0.0018$). In contrast to the frequency-specific decline in sensitivity observed in *P. californicus*, the qualitative shape of the *P. leucopus* audiogram was consistent among age groups (Fig. 1D), indicating global, albeit small, threshold shifts with age.

Age-related Changes to ABR Waveform Amplitudes

ABR morphology was similar among *M. musculus* and *Peromyscus* mice, with 4–5 peaks observed in the evoked response (Fig. 3), although wave V was often difficult to reliably detect due to its relatively small and highly variable amplitude. The amplitudes of ABR wave I were notably larger in *P. leucopus* relative to *P. californicus* and especially to *M. musculus*. Wave I amplitudes evoked by
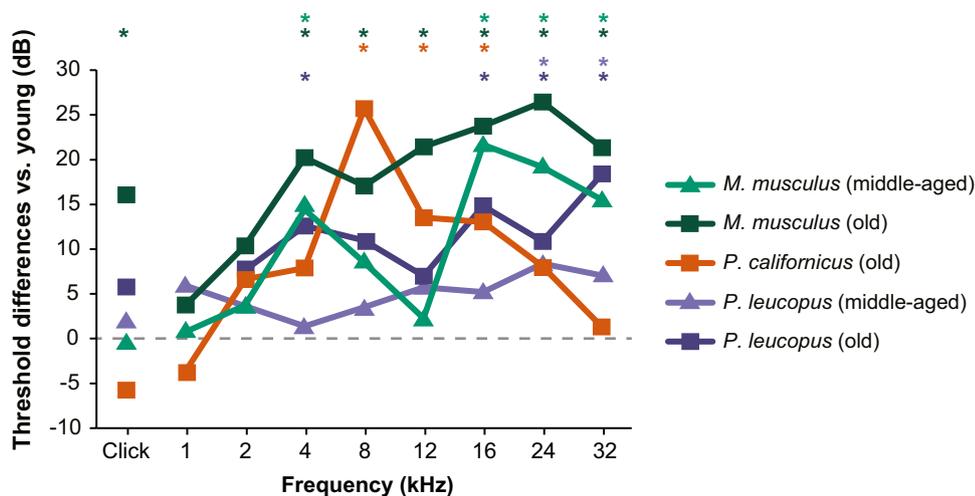
Fig. 2 Age-related changes to hearing sensitivity across species. Threshold differences are calculated as the difference in mean detection thresholds measured in middle-aged and old animals relative to mean thresholds measured in young individuals for each frequency. Asterisks indicate significant deviations from mean young thresholds ($p < 0.05$)
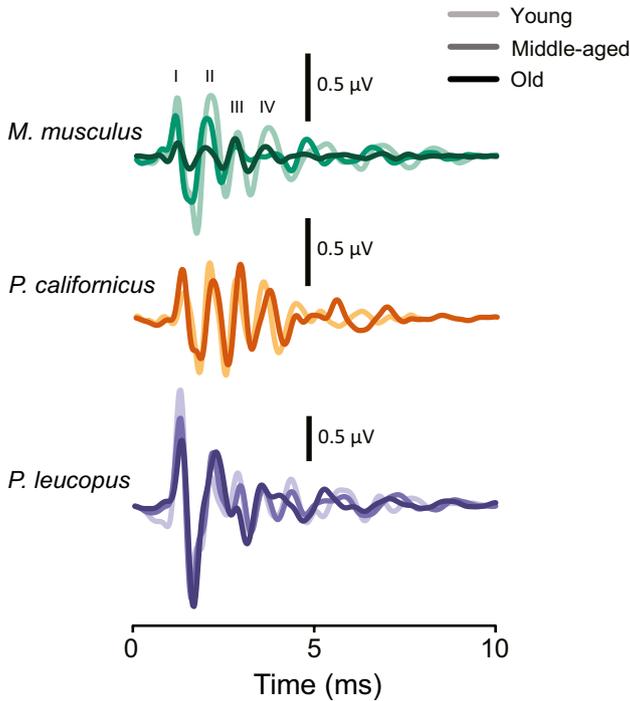
Fig. 3 ABR wave morphology across age groups in three rodent species. Representative ABR traces recorded in individual young, middle-aged, and old *Mus musculus* and *Peromyscus leucopus*, and young and old *P. californicus* in response to click stimuli presented at 90 dB re 20 µPa. ABRs are scaled to a stimulus onset at 0 ms. Note scale bar variation among species due to species-specific differences in response amplitudes

click, 8 kHz, and 24 kHz stimulation in young, middle-aged, and old *P. leucopus* were twice the amplitude of those recorded in age-matched *M. musculus* (Table 2, Supplementary Tables S30 and S47).

Age-related changes to hearing in *M. musculus* were indicated by reduced amplitudes (Fig. 4, Table 2) and increased latencies of the ABR waves, even in strains such as CBA/CaJ that do not show early-onset hearing loss. The CBA/CaJ strain tested in the present study demonstrated a strong relationship between age group and the peak-to-trough amplitudes of ABR waves I–IV recorded in response to a 90 dB click stimulus (LMM: wave I: $F_{(2,34.69)} = 25.04$, $p = 1.86E\text{-}7$; wave II: $F_{(2,34.58)} = 13.47$, $p = 4.71E\text{-}5$; wave III: $F_{(2,34.50)} = 9.474$, $p = 5.25E\text{-}4$; wave IV: $F_{(2,35)} = 21.19$, $p = 9.33E\text{-}7$). Old *M. musculus* had smaller click-evoked ABR wave amplitudes for waves I-IV relative to young individuals ($p < 0.001$ for all pairwise comparisons, see Supplementary Table S15). Wave I showed a marginally significant effect of the interaction of age and sex in *M. musculus* (LMM: $F_{(2,34.69)} = 3.32$, $p = 0.048$) in which click stimuli evoked waves of comparable amplitude in young and middle-aged females ($p = 1.000$), whereas middle-aged males had smaller amplitude responses relative to young males ($p = 0.0284$). Wave IV appeared most susceptible to age-related amplitude reductions: middle-aged individuals had significantly reduced click-evoked wave IV amplitudes relative to young animals ($p = 0.0035$), and wave IV amplitudes recorded from old individuals were smaller than both young ($p < 0.0001$) and middle-aged ($p = 0.0156$) individuals; see Fig. 4.

M. musculus showed significant age-effects on ABR wave amplitudes in response to 8 kHz tone stimuli (LMM: wave I: $F_{(2, 35)} = 13.19$, $p = 5.37E\text{-}5$; wave II: $F_{(2, 35)} = 14.17$, $p = 3.10E\text{-}5$; wave III: $F_{(2, 35)} = 14.79$, $p = 2.21E\text{-}5$; wave IV: $F_{(2, 35)} = 11.86$, $p = 1.16E\text{-}4$). In general, young individuals had larger amplitude responses evoked by 8 kHz tones relative to middle-aged individuals and old individuals (see Supplementary Table S33 for pairwise

TABLE 2

Age-related changes to the amplitudes of waves I-IV of the click-evoked auditory brainstem response of *Mus musculus*, *Peromyscus californicus*, and *P. leucopus*

Species	Age group	Sample size	Wave I amplitude (µV)	Wave II amplitude (µV)	Wave III amplitude (µV)	Wave IV amplitude (µV)
M. musculus	Young	13	8.99 (±0.87)	5.80 (±0.76)	5.15 (±0.57)	3.55 (±0.34)
M. musculus	Middle-aged	12	6.46 (±0.62)	4.28 (±0.39)	2.84 (±0.56)	2.15 (±0.31)
M. musculus	Old	16	2.52 (±0.51)	1.90 (±0.34)	1.81 (±0.42)	1.03 (±0.19)
P. californicus	Young	6	7.99 (±0.85)	5.19 (±1.02)	4.46 (±0.79)	5.47 (±1.08)
P. californicus	Old	6	6.77 (±1.25)	5.62 (±0.68)	3.57 (±0.43)	2.70 (±0.55)
P. leucopus	Young	6	18.65 (±2.16)	5.54 (±0.79)	5.11 (±1.27)	6.28 (±1.13)
P. leucopus	Middle-aged	21	11.50 (±0.92)	5.28 (±0.51)	4.08 (±0.42)	3.49 (±0.37)
P. leucopus	Old	5	8.89 (±2.91)	3.72 (±0.82)	2.43 (±0.89)	2.36 (±0.43)

Values represent mean peak-to-trough amplitudes (±standard error of the mean) of each wave of the ABR evoked by a click stimulus presented at 90 dB re 20µPa
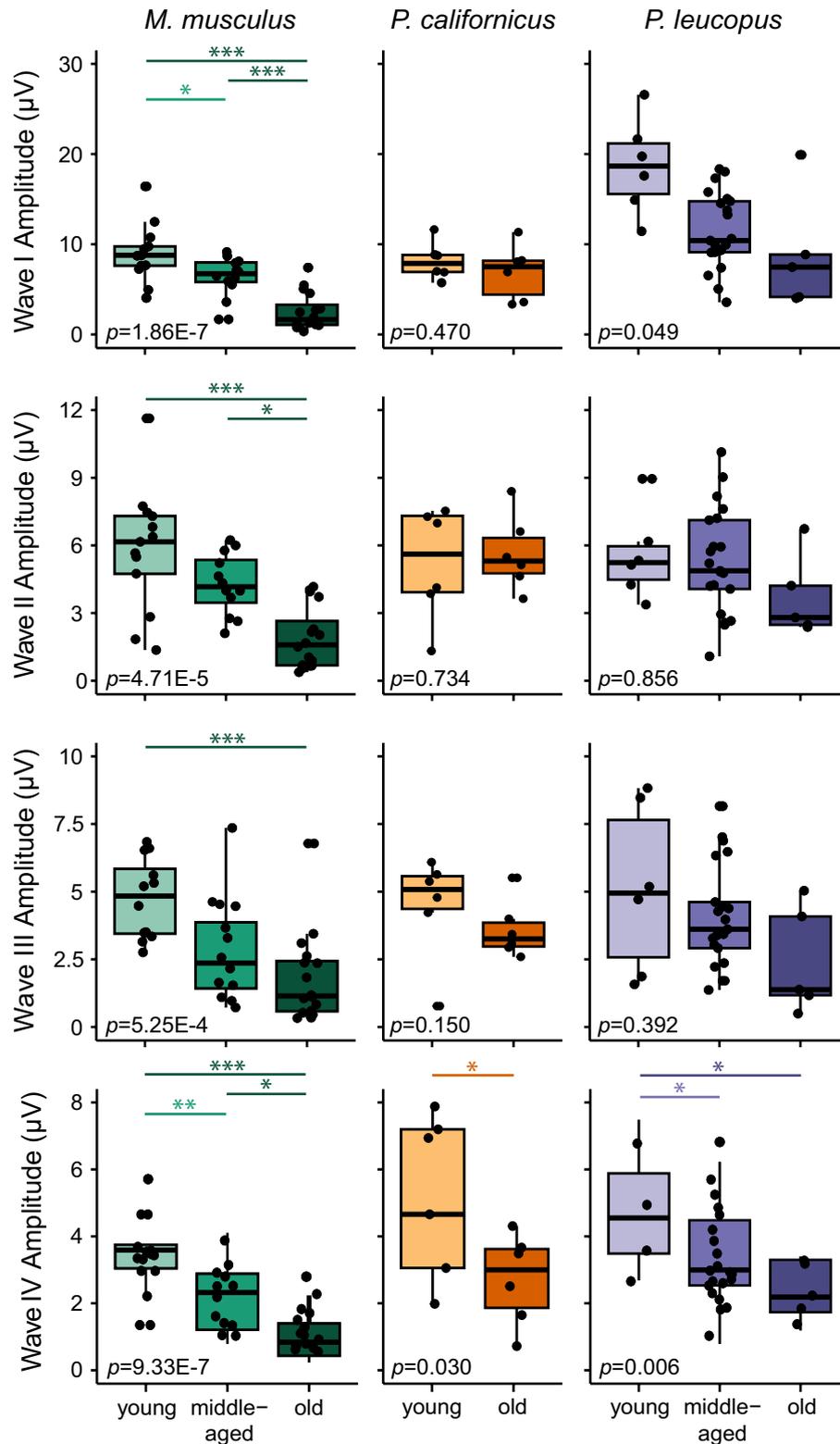
Fig. 4 Age-related differences in amplitudes of ABR waves I–IV recorded in three rodent species. Data represent responses evoked by a 90 dB re 20 μPa click stimulus among age groups of *M. musculus* (young, $n=13$; middle-aged, $n=12$; old, $n=16$), *P. californicus* (young, $n=6$; old, $n=6$), and *P. leucopus* (young, $n=6$; middle-aged, $n=21$; old, $n=5$). *p* values represent significance of the main effect of age group on ABR wave amplitudes. Asterisks indicate significant differences between age groups based on post hoc pairwise comparisons (*$p<0.05$; **$p<0.01$; ***$p<0.001$)

comparisons of wave I–IV amplitudes). We observed similar trends in age-related amplitude decline among *M. musculus* in response to stimulation by 24 kHz tones. ABR waves I and IV both showed significant effects of age (LMM: wave I: $F_{(2, 33.56)} = 23.44$, $p = 4.27\text{E-}7$; wave IV: $F_{(2, 33)} = 15.46$, $p = 1.83\text{E-}5$) and the interaction of age and sex (LMM: wave I: $F_{(2, 33.56)} = 3.56$, $p = 0.040$; wave IV: $F_{(2, 33)} = 4.82$, $p = 0.015$). The amplitudes of waves I and IV evoked by 24 kHz tones were comparable among young males and females (wave I: $p = 0.133$; wave IV: $p = 0.121$); however, there was a sex difference in the magnitude of decline among males and females in which middle-aged and old males showed significant amplitude reductions compared to young males for waves I ($p = 0.0003$ for both comparisons) and IV (middle-aged, young $p = 0.0034$; old, young $p = 0.0008$). In contrast, wave I and IV amplitudes from young and middle-aged females did not differ significantly (wave I: $p = 0.312$; wave IV: $p = 1.000$). Waves II and III of the 24 kHz tone-evoked ABR showed age-related declines that did not vary significantly among sexes in which young individuals had larger amplitude responses relative to middle-aged (wave II: $p = 0.0004$, wave III: $p = 0.0017$) and old individuals (wave II: $p = 0.0001$, wave III: $p = 0.0001$).

Age-related changes to click-evoked ABR wave amplitudes were smaller in *Peromyscus* species relative to *Mus* (Fig. 4). We recovered a significant effect of age group on click-evoked wave IV amplitudes in *P. californicus* (LMM: $F_{(1,9)} = 6.65$, $p = 0.0297$) in which young individuals had larger amplitude responses than old ($p = 0.041$); however, individual response amplitudes in young *P. californicus* were variable, and we observed no other age-related changes to click-evoked wave amplitudes in this species (Table 2). We observed no statistically significant differences in the click-evoked amplitudes of waves I–III across sex or age group in *P. californicus* nor in the click-evoked amplitudes of waves II and III in *P. leucopus* (Supplementary Tables S17 and S20).

We observed a marginally significant effect of age (LMM: $F_{(2,20.06)} = 3.52$, $p = 0.049$) on click-evoked ABR wave I amplitudes recorded in *P. leucopus*; however, post hoc pairwise comparisons did not recover significant differences among age groups when both sexes were pooled. Although sex was not a significant main effect on click-evoked wave amplitudes, the inclusion of sex in post-hoc comparisons revealed that variation in wave I amplitude appears driven by large responses recorded from young females, especially relative to middle-aged ($p = 0.016$) and old males ($p = 0.037$) that generally had the smallest amplitude responses. Within-sex comparisons of click-evoked response amplitudes were not significantly different across age groups, e.g., amplitudes measured in young females were not significantly greater than those of middle-aged ($p = 0.477$) or old females ($p = 0.934$). Click-evoked amplitudes of wave IV showed age-related changes in *P. leucopus* ($F_{(2,28)} = 6.08$, $p = 0.006$),

where young individuals had higher amplitude responses to clicks relative to both middle-aged ($p = 0.0293$) and old individuals ($p = 0.0205$).

P. californicus and *P. leucopus* showed no significant age-related differences observed in ABR wave amplitudes evoked by 8 or 24 kHz stimulation in (Supplementary Tables S35, S37, S52 and S55). Although we found marginally significant effects of age on *P. leucopus* wave III amplitudes evoked by 8 kHz tones (LMM: $F_{(2, 28)} = 3.44$, $p = 0.046$), post hoc pairwise comparisons did not identify significant differences between response amplitudes recorded from young individuals and middle-aged ($p = 0.47$) or old individuals ($p = 0.057$).

Age-related Changes to ABR Waveform Latencies

The latencies of waves I–IV of the click-evoked response in *M. musculus* were strongly influenced by age (Fig. 5, Table 3). Old individuals had significantly longer latencies to each ABR wave relative to both young and middle-aged individuals ($p < 0.05$ for all comparisons; see Supplementary Table S24). Compared to click-evoked ABRs recorded from young *M. musculus*, responses from old individuals showed average delays ranging from 0.26 to 0.28 ms for ABR waves I and II to greater than 0.50 ms for later waves. We observed no significant variation in *M. musculus* ABR wave latencies evoked in response to 8 kHz tones (Supplementary Table S41), although there were significant latency differences in ABR wave I evoked by 24 kHz stimulation ($F_{(2, 6.41)} = 8.55$, $p = 0.0155$) in which old individuals had greater response delays than both young ($p = 0.0387$) and middle-aged individuals ($p = 0.0225$). For all species tested, model selection procedures did not favor the inclusion of sex as a fixed effect for statistical comparison of wave latencies evoked by click, 8 kHz, or 24 kHz stimulation, indicating that sex was not the best predictor for intraspecific changes in response latency.

There was no significant relationship between age group and latencies for click-evoked ABR waves I–IV in *P. californicus*. Although *P. californicus* hearing thresholds showed their greatest age-related shifts in response to 8 kHz tones, we observed no significant differences in ABR latencies for waves I–IV (Supplementary Table S43). *P. californicus* response latencies to 24 kHz stimulation were similarly robust to age effects (Supplementary Table S61).

P. leucopus showed age-related latency shifts for wave I of the click-evoked ABR ($F_{(2,8.66)} = 0.17$, $p = 0.0009$) in which middle-aged individuals had longer latencies relative to young individuals ($p = 0.0235$), and old individuals had longer latencies than both middle-aged ($p = 0.0141$) and young ($p < 0.0001$) individuals. Wave I latencies evoked by 8 kHz tones were also influenced by age ($F_{(2, 26.38)} = 10.00$, $p = 0.0006$) in which young individuals had shorter latencies than both middle-aged ($p = 0.0097$)
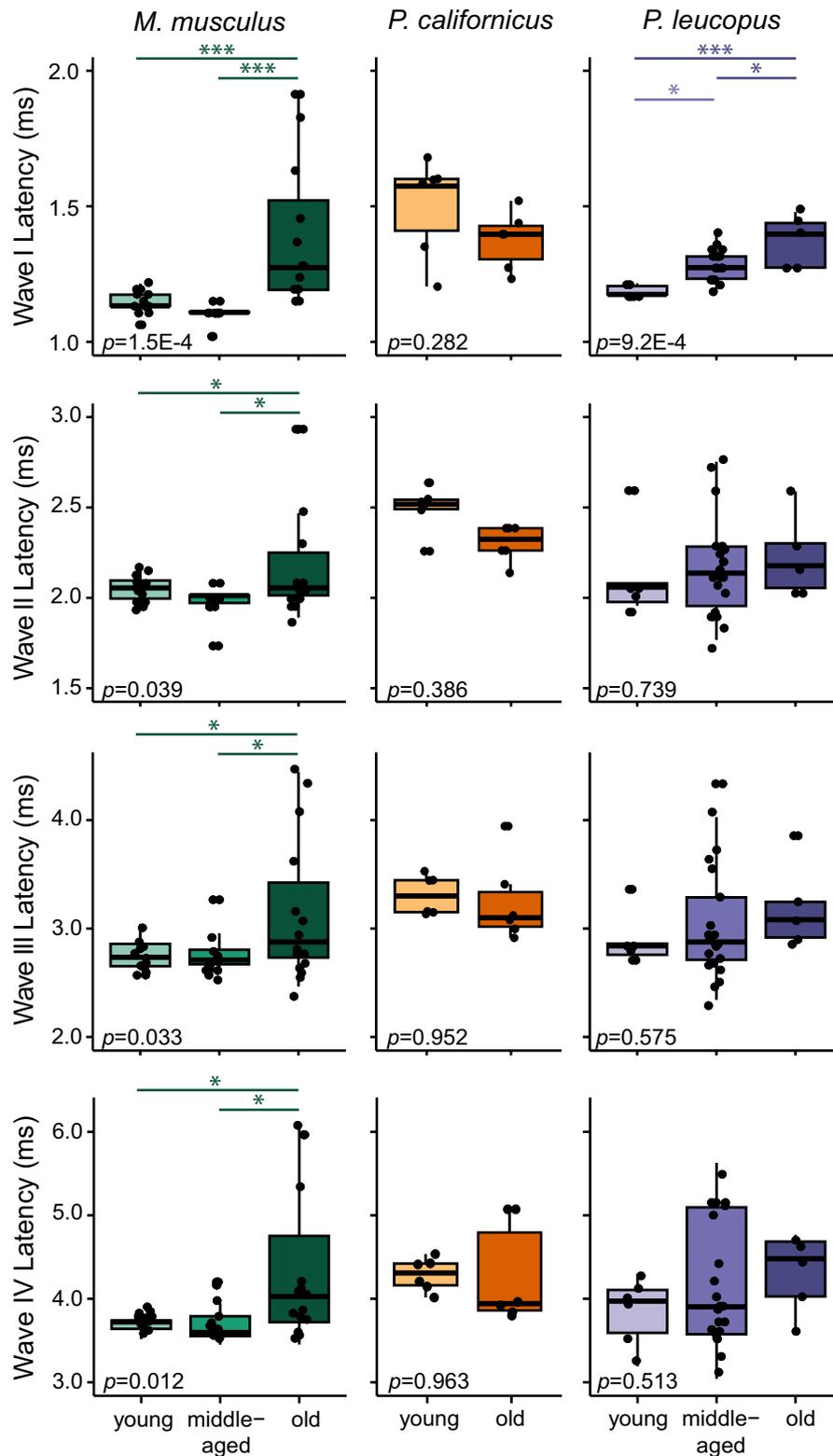
FIG. 5 Age-related differences in ABR wave latencies in three rodent species. Data represent latencies of ABR waves I–IV evoked by a 90 dB re 20 µPa click stimulus among age groups of *M. musculus* (young, $n=13$; middle-aged, $n=12$; old, $n=16$), *P. californicus* (young, $n=6$; old, $n=6$), and *P. leucopus* (young, $n=6$; middle-aged, $n=21$; old, $n=5$). p values represent significance of the main effect of age group on ABR wave latencies. Asterisks indicate significant differences between age groups based on post hoc pairwise comparisons (*$p<0.05$; ***$p<0.001$)

TABLE 3

Age-related changes to the latencies of waves I–IV of the click-evoked auditory brainstem response of *Mus musculus*, *Peromyscus californicus*, and *P. leucopus*

Species	Age group	Sample size	Wave I latency (ms)	Wave II latency (ms)	Wave III latency (ms)	Wave IV latency (ms)
M. musculus	Young	13	1.15 (±0.01)	2.06 (±0.02)	2.77 (±0.04)	3.70 (±0.03)
M. musculus	Middle-aged	12	1.11 (±0.01)	1.99 (±0.02)	2.77 (±0.05)	3.70 (±0.07)
M. musculus	Old	16	1.43 (±0.08)	2.32 (±0.11)	3.26 (±0.18)	4.27 (±0.25)
P. californicus	Young	6	1.50 (±0.08)	2.49 (±0.05)	3.31 (±0.07)	4.23 (±0.08)
P. californicus	Old	6	1.38 (±0.04)	2.30 (±0.04)	3.25 (±0.16)	4.22 (±0.25)
P. leucopus	Young	6	1.19 (±0.01)	2.11 (±0.09)	2.90 (±0.09)	3.84 (±0.17)
P. leucopus	Middle-aged	21	1.29 (±0.01)	2.18 (±0.06)	3.04 (±0.11)	4.17 (±0.17)
P. leucopus	Old	5	1.37 (±0.04)	2.24 (±0.10)	3.19 (±0.17)	4.31 (±0.22)

Values represent mean latencies (±standard error of the mean) of the ABR waves evoked by a click stimulus presented at 90 dB re 20µPa. Latencies are calculated as time to maximum amplitude of each wave relative to click onset time

and old individuals ($p = 0.0022$). We observed significant age-effects on the latencies of waves I ($F_{(2,25.88)} = 4.65$, $p = 0.0188$) and III ($F_{(2,15.72)} = 5.51$, $p = 0.0154$) evoked by 24 kHz stimulation; however, post hoc pairwise comparisons did not reveal any significant trends among age groups (Supplementary Table S64).

DISCUSSION

In the present study, we used ABR recordings to evaluate hearing sensitivity across the lifespan of three rodent species, the house mouse (*Mus musculus*), and two deer mice species from the genus *Peromyscus*: *P. californicus* and *P. leucopus*. Our goal was to compare the trajectory of age-related hearing loss (ARHL) in these two relatively long-lived species to that of the CBA/CaJ mouse strain, a laboratory model commonly used in auditory neuroscience. In general, ABR-derived detection thresholds showed similar frequency ranges of sensitivity among species in which best hearing was for 8–16 kHz sounds. Although *P. californicus* and *P. leucopus* had lower thresholds for frequencies below 8 kHz, we did not observe enhanced sensitivity to frequencies below 2 kHz. In this regard, our results are not fully consistent with previous reports of greater low-frequency sensitivity among *Peromyscus* mice relative to *Mus* (Ralls 1967). However, mammals with small heads are unable to generate physiologically useful directional cues from low- frequency sounds for which binaural timing and level differences are minimal. Consequently, rodents must rely on high- frequency sounds for directional hearing (Lauer et al. 2011, 2018). Thus, poor low-frequency sensitivity in small rodents has been attributed to selective pressures related to the lack of interaural cues for sound localization in the horizontal plane (Heffner and Heffner 1992; Heffner et al. 2001). In

this context, the comparable sensitivity to low frequencies observed in *Peromyscus* and *Mus* in the present study is unsurprising.

Sensitivity to Low Frequencies May Mediate Long-distance Communication in Peromyscus

In addition to sound localization, small rodents use high-frequency sounds to mediate social behaviors including courtship, mother–pup interactions, alarm calling, aggression, and territoriality. *P. californicus* is a monogamous, biparental species that produces USVs with 1–3 syllables (Kalcounis-Rueppell et al. 2006, 2010; Miller and Engstrom 2012) with no sex differences observed in the structure or context of vocalizations (Briggs and Kalcounis-Rueppell 2011; Rieger and Marler 2018). These vocalizations range from approximately 10–21 kHz with the majority of acoustic energy centered around 18 kHz (Miller and Engstrom 2012). Although *P. californicus* are sensitive to frequencies within range of their USVs, we observed peak sensitivity to 8 kHz tones in young individuals (Fig. 6). Lower frequencies are more effectively transmitted across longer distances, and *P. californicus* has been observed to emit relatively loud, low-frequency sustained vocalizations when calling in isolation or at a distance from a conspecific (Briggs and Kalcounis-Rueppell 2011). Auditory sensitivity to low frequencies in this species therefore could enable long distance detection of acoustic signals, whereas high-frequency call components may be more behaviorally salient at short distances. Similar findings have been reported in grasshopper mice (*Onychomys leucogaster*), in which auditory sensitivity appears more strongly tuned to the low-frequency components of their long-distance calls (Green et al. 2019).
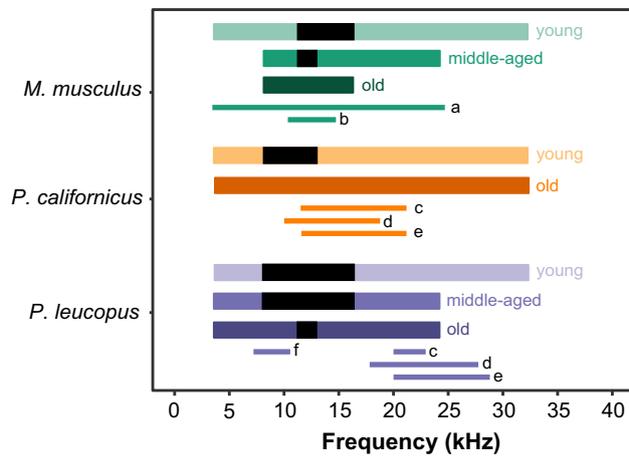
Fig. 6 Ranges of best sensitivity across age groups for three rodent species. Colored bars indicate range of frequencies that can be detected at ≤60 dB re 20 μPa for each age group; black bars indicate frequency ranges detectable at ≤30 dB re 20 μPa. Lines indicate frequency ranges reported for calls emitted by *M. musculus* and *Peromyscus* mice in which the majority of spectral energy overlaps with frequency ranges of best sensitivity measured in this study. *M. musculus* calls include **a** aversive "squeaks" that are referred to as "broadband vocalizations" or "low frequency harmonic vocalizations" (Lupanova and Egorova 2015; Finton et al. 2017) and **b** mid-frequency release calls emitted during restraint (Grimsley et al. 2016). Calls emitted by *Peromyscus* mice include **c** alarm calls (García-Navas and Blumstein 2016), stereotypic vocalizations of **d** females and **e** males (Miller and Engstrom 2012), and **f** intraspecific aggressive vocalizations (Houseknecht 1968)

P. leucopus are highly territorial and emit USVs that range from 17 to 28 kHz with peak frequencies of 24–25 kHz (Miller and Engstrom 2012). It is notable, however, that this species' best sensitivity appears strongly mis-matched to the frequency components of their USVs (Fig. 6). Agonistic vocalizations in rodents, such as those associated with distress or defensive behaviors, are generally broadband with low-frequency harmonics between 2 and 30 kHz (Portfors 2007; Ehret 2013; Rieger and Marler 2018). Although few studies have characterized distress or aggressive vocalizations in *P. leucopus*, this species has been observed to emit two call types during agonistic encounters: "chits," which are broadband signals extending into low (< 15 kHz) frequencies and "barks" that resemble inverse chevrons with spectral energy from approximately 6–11 kHz (Houseknecht 1968). These agonistic vocalizations more closely match peak auditory sensitivity of this species, suggesting that lower frequency sounds are important for mediating aversive behaviors in *P. leucopus*. This, combined with data from *P. californicus* described above, provides evidence that good sensitivity to frequencies from 8 to 12 kHz in these species may enhance detection of the low-frequency components of acoustic signals that are more likely to propagate at greater distances.

Peromyscus Mice are Resistant to Age-related Hearing Loss

In *Peromyscus* mice, the decline in auditory thresholds across different age groups was lower than threshold shifts observed in *M. musculus* (CBA/CaJ). Middle-aged *P. leucopus* showed hearing loss at 24 and 32 kHz, but no change in sensitivity from 1 to 16 kHz. In old *P. leucopus*, auditory threshold increases progressed to all frequencies above 4 kHz. While this result is in line with the expected course of ARHL in mammals (Bowl and Dawson 2019), the threshold shifts observed in *P. leucopus* were significantly smaller than those measured in old CBA/CaJ mice. Old *P. californicus* showed a reduction in sensitivity to mid-range frequencies (8–16 kHz) that was greatest for 8 kHz tones to which young adults show peak sensitivity (within the frequency range evaluated in this study). We found no evidence for high-frequency (> 16 kHz) hearing loss, suggesting that ARHL in *P. californicus* is frequency-specific and primarily affects the region of best sensitivity. This result differs from what has been observed in other rodents including house mice, grasshopper mice, rats, and gerbils, as well as in humans, where ARHL usually first impairs high-frequency sensitivity and then progresses to other frequencies (Henry et al. 1980; Mills et al. 1990; Bowl and Dawson 2019; Kobrina et al. 2020, 2021a; Vicencio-Jimenez et al. 2021). While the large variation we observed in the ABR thresholds of old *P. californicus* could be influencing this result, it is relevant that variability in the ABR of old animals has been previously reported to precede changes in thresholds detected with behavioral measures (Kobrina et al. 2020).

The minimal effects of age on the *Peromyscus* audiogram are complemented by reduced age-related changes to ABR wave amplitudes and latencies in these species relative to *Mus*. *P. leucopus* had notably large wave amplitudes that, except for wave IV, showed non-significant declines with age and generally remained larger than those recorded in *P. californicus* and *M. musculus* across all age groups. Although ABR wave I of *P. leucopus* demonstrated a gradual increase in latency with age, latency shifts from young to old age were less extreme in this species, with onset of the ABR delayed on average by 0.18 ms relative to mean delays of 0.28 ms in old *M. musculus*. ABRs recorded from *P. californicus* were similarly robust to age-related declines and demonstrated no significant changes to ABR wave metrics. Thus, the absence of significant changes to ABR latency and amplitude in old *Peromyscus* mice suggests that these species may be resistant to ARHL. Given the dearth of previous studies of the *Peromyscus* auditory system, it is difficult to speculate about the mechanisms that account for the resistance to ARHL that we observed in *P. leucopus* and *P. californicus*. However, one possibility for this healthier auditory aging could be the reduced production of mitochondrial reactive oxygen species and enhanced activity of antioxidant

enzymes that have been described in this genus (Sohal et al. 1993; Csiszar et al. 2007).

The gradual accumulation of oxidative damage with age is hypothesized to underlie many pathologies related to senescence, including in the cochlea and central auditory pathway (Seidman et al. 2004; Jiang et al. 2007; Ohlemiller and Frisina 2008; Du et al. 2015; Fetoni et al. 2015). The prevalence of ARHL is highly variable in humans, and, although the factors underlying how the human hearing loss phenotype is expressed are still being elucidated, degeneration of the cochlear outer hair cells and the stria vascularis are the most common features of ARHL in humans (Schuknecht and Gacek 1993; Gates and Mills 2005; Nelson and Hinojosa 2006) and rodents (Schulte and Schmiedt 1992; Heeringa and Köppl 2019; Kobrina et al. 2020). Susceptibility to oxidative stress is also individually variable among humans, and the correlation between ARHL and oxidative stress markers (e.g., inflammation and cellular apoptosis) is often confounded by variable noise exposure histories and other co-morbidities such as vascular disease (Yamasoba et al. 2013). However, age-related damage to the stria and other cellular structures of the cochlea may be exacerbated by increased activity of reactive oxygen species (Ohlemiller and Frisina 2008; Menardo et al. 2012; Yamasoba et al. 2013) and the reduction of cochlear antioxidant enzyme activity that naturally declines with age in humans (Hosokawa et al. 2018) and in rodent models (Staecker et al. 2001; Jiang et al. 2007; Menardo et al. 2012). Mutant *Mus* strains that lack genes to encode antioxidant enzymes have more severe age-related degeneration of hair cells and the stria vascularis and demonstrate early onset hearing loss relative to wild-type mice (McFadden et al. 1999; Keithley et al. 2005). Moreover, antioxidant supplementation interventions can prevent the onset of symptoms associated with ARHL in animal models (Le and Keithley 2007; Ding et al. 2016), although human outcomes are mixed (reviewed in Tavanai and Mohammadkhani 2017). The longevity of *Peromyscus* mice is correlated with their cellular resistance to oxidative stress, which protects against age-related degeneration of vascular and skeletal tissue (Csiszar et al. 2007; Ungvari et al. 2008; Shi et al. 2013) and may similarly preserve cochlear function to delay onset of ARHL.

CONCLUSIONS AND FUTURE DIRECTIONS

Our results demonstrate that *Peromyscus* mice have great potential as additional mammalian models for auditory studies, particularly for studies of age-related changes to auditory processing. The hearing range of the *Peromyscus* species in the present study broadly overlaps with that of *Mus musculus*, although with greater sensitivity to frequencies from 2 to 12 kHz. Physiological assessment of auditory sensitivity across the lifespan of *P. californicus* and *P. leucopus* revealed differences in the pattern of ARHL relative to

Mus in which both *Peromyscus* species retained good hearing thresholds and comparable response amplitudes and latencies into old age. Further anatomical characterization of the cochlea, auditory nerve, and central auditory system is necessary to understand the mechanisms that may underlie resistance to ARHL among long-lived *Peromyscus* species. The longevity of *Peromyscus* species provides the additional opportunity to develop behavioral assays of hearing, many of which require lengthy training periods that can be difficult to achieve using short-lived animals such as *Mus*. Future studies should also characterize temporal processing, masking and frequency selectivity, sound discrimination, and spatial hearing in *Peromyscus* to establish the utility of these animals in investigating more complex hearing abilities and their underlying mechanisms.

Rodent models offer great insights into the mechanistic bases of ARHL that can be difficult to evaluate in human subjects; however, the genetic homogeneity of inbred *Mus* strains used in many studies is not representative of free-living animals, including humans, making it challenging to translate findings to heterogeneous populations. Although natural audiometric variation within a species is best represented by individuals sourced from wild populations, the *Peromyscus* Genetic Stock Center (University of South Carolina) maintains carefully outbred, genetically diverse colonies that facilitate the accessibility of these species for laboratory-based research. Outbred and wild-derived *Mus* strains are also available, but many of these strains exhibit early-onset hearing loss, e.g., CD-1 (Shone et al. 1991) and Black Swiss mice (Drayton and Noben-Trauth 2006), or are difficult to maintain in the lab, e.g., CAST/EiJ (Boggess et al. 1999). Additionally, the *Peromyscus* genome project provides key sequence data that will enhance the development of genomic tools for these species (Ramsdell et al. 2008; Long et al. 2019). Although ARHL is common among aging humans, the nature and etiology of hearing impairment is multi-faceted and highly variable among individuals. *Peromyscus* species represent a valuable alternative model species that will complement hearing studies using *Mus* and provide the opportunity to explore a range of phenotypes that may better represent the human condition.

Data availability Data will be made available upon request.

Declarations

Ethics Approval All experiments were approved by the Johns Hopkins University Animal Care and Use Committee.

Conflict of Interest The authors declare no competing interests.

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