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Research Paper

Age-related and noise-induced hearing loss alters grasshopper mouse (*Onychomys*) vocalizations

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ABSTRACT

Age-related and noise-induced hearing loss disorders are among the most common pathologies affecting Americans across their lifespans. Loss of auditory feedback due to hearing disorders is correlated with changes in voice and speech-motor control in humans. Although rodents are increasingly used to model human age- and noise-induced hearing loss, few studies have assessed vocal changes after acoustic trauma. Northern grasshopper mice (*Onychomys leucogaster*) represent a candidate model because their hearing sensitivity is matched to the frequencies of long-distance vocalizations that are produced using vocal fold vibrations similar to human speech. In this study, we quantified changes in auditory brainstem responses (ABRs) and vocalizations related to aging and noise-induced acoustic trauma. Mice showed a progressive decrease in hearing sensitivity across 4–32 kHz, with males losing hearing more rapidly than females. In addition, noise-exposed mice had a 61.55 dB SPL decrease in ABR sensitivity following a noise exposure, with some individuals exhibiting a 21.25 dB recovery 300–330 days after noise exposure. We also found that older grasshopper mice produced calls with lower fundamental frequency. Sex differences were measured in duration of calls with females producing longer calls with age. Our findings indicate that grasshopper mice experience age- and noise- induced hearing loss and concomitant changes in vocal output, making them a promising model for hearing and communication disorders.

1. Introduction

Acoustic communication relies on the dual ability to produce and hear vocalizations of oneself and others. Humans and other animals can monitor and alter vocal production by relying on auditory and somatosensory feedback (Brainard and Doupe, 2000; Smotherman, 2007; Tschida and Mooney, 2012). In vocal learning species, auditory feedback is critical for learning and altering communication signals (Brainard and Doupe, 2000; Lane et al., 2007; Nordeen and Nordeen, 1992; Osmanski and Dooling, 2009; Tschida and Mooney, 2012), although some evidence suggests that non-vocal learning species may similarly rely on auditory feedback for vocal production (Arriaga and Jarvis 2013; Arriaga et al., 2012; Eliades and Wang, 2008; Hubka et al., 2015; Shipley et al., 1988). Age-related, noise-induced, and neurodegenerative changes to hearing can disrupt the auditory feedback loop to affect voice and communication ability, decreasing quality of life in humans (Dalton et al., 2003; Harel et al., 2004; Homans et al., 2017; Li-Korotky, 2012; Roy et al., 2007). Although rodents are emerging models of age-related hearing loss (ARHL) and noise-induced hearing loss (NIHL), few studies have assessed if vocal production disorders correlate with impaired hearing (but see Arriaga et al., 2012). Examining interactions between ARHL, NIHL, and changes in voice production in a mouse model using longitudinal studies can improve our understanding of this process in humans.

Laboratory mice are popular animal models for human hearing loss disorders due to similarities in physiology, ease of genetic manipulation, and tractability (reviewed by Dent et al., 2018; Ohlemiller, 2018). For example, various genetically engineered mouse strains exhibit different levels of hearing deficits and variable susceptibility to noise (Davis et al., 2001; Erway et al., 1996; Kane et al., 2012; Ohlemiller et al., 2016). In general, mice lose hearing similarly to humans starting with high frequencies and progressing to lower frequencies (Henry, 2004;

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Huang and Tang, 2010), with the greatest degree of ARHL occurring in the last third of their lifespan (Kobrina and Dent, 2019). ARHL effects exist for detection of pure tones (Henry, 2004; Kobrina and Dent, 2019; Zheng et al., 1999) and ultrasonic vocalizations (USVs; Kobrina and Dent, 2016), though both mice and humans retain their ability to hear communication signals longer into their lifespan than pure tones (Huang and Tang, 2010; Kobrina and Dent, 2016; Kobrina et al., 2020).

In addition to ARHL, most humans and other animals are exposed to various levels of noise in their environment. In humans, noise exposure often leads to hearing loss and deficits in speech comprehension (Liberman, 2017; Liberman et al., 2016; reviewed by Moore, 2016). Similarly, mice experience decreased hearing abilities, increased hair cell loss, and cell death in the auditory brainstem and auditory cortex after high-level noise exposure (reviewed by Ohlemiller, 2006; Fröhlich et al., 2017). As in humans, mice may partially recover from hearing loss depending on the level of noise exposure (Amanipour et al., 2018; Lin et al., 2009).

In contrast to the large body of literature on ARHL and NIHL in humans and rodents, few studies have assessed the impacts of hearing loss on vocal production. In humans, voice quality is linked to auditory feedback; individuals who are congenitally deaf or have severe hearing loss exhibit altered speech (Coelho et al., 2015; Higgins et al., 2003, 2005; Nicholas and Geers, 2006). In addition, age-related sensorineural hearing loss and deafness are associated with increased fundamental frequency (F_0 hereafter), decreased voice clarity (as measured through harmonic-to-noise ratio; HNR), and lack of intensity control (Baken, 2005; Benjamin, 1982; Binnie et al., 1982; Blamey et al., 2001; Coelho et al., 2015; dos Santos Baraldi et al., 2007; Ferrand, 2002; Mora et al., 2012; Waldstein, 1990). Such speech deficiencies may arise via auditory feedback-related effects that impact laryngeal function or vocal tract-related effects that alter coordinated oral and pharyngeal movements (Lane and Perkell, 2005; Perkell et al., 2000). Hearing loss and deafness may similarly impact vocal production in non-vocal learning mammals (Basken et al., 2012). For example, deafened cats produced louder calls of variable fundamental frequency and longer duration compared to littermate controls (Hubka et al., 2015; Shipley et al., 1988). While the necessity of auditory feedback for learning and production of USVs in mice is controversial (Hammerschmidt et al., 2012), physiological evidence indicates that the laryngeal motor cortex receives inputs from the thalamus and the secondary auditory cortex, a network similar to the auditory feedback loop in vocal-learning species (Arriaga and Jarvis, 2013). Deafened mice produce spectrally distorted and noisy USVs compared to controls, with congenitally deaf mice having a simpler repertoire than animals with acoustic experience (Arriaga and Jarvis, 2013; Arriaga et al., 2012). Together, these findings suggest that the use of auditory feedback may be a generalized mammalian process for vocal control.

The main constraint of modeling human vocal production in laboratory rodents is the highly diverse USV repertoire with no standard categorization method (Johnson et al., 2015) and the physiologically unique aerodynamic whistle mechanism of USV production compared to human speech (Riede et al., 2017). Exploring non-model organisms may help researchers understand complex biomedical questions in species with unique traits (Christie and Eberl, 2014; Peter et al., 2017). Grasshopper mice (genus *Onychomys*) represent a unique model for human voice production and hearing loss. These mice inhabit arid environments throughout the western United States and northern Mexico (Egoscue, 1960) and are known for their aggression, predatory lifestyle, and large solitary home ranges (Bailey and Sperry, 1929; Ruffer, 1968; Stapp, 1999). Grasshopper mice produce both USVs and loud, audible, stereotyped advertisement calls (Egoscue, 1960; Hafner and Hafner, 1979; Miller and Engstrom, 2012; Pasch et al., 2016; Pasch et al., 2017). Advertisement vocalizations are characterized by a peak F_0 of 10–12 kHz with several harmonic overtones that are produced by an airflow-induced tissue vibration mechanism, a mechanism similar to production of human speech (Pasch et al., 2017) (Fig. 1). These findings indicate that the laryngeal and vocal tract physiology in grasshopper mice is more relevant to humans than the physiology of laboratory rodents that produce ultrasonic vocalizations using an aerodynamic whistle mechanism (Riede, 2013; Riede et al., 2017). In addition, grasshopper mice have a broad peripheral auditory sensitivity with increased sensitivity for their advertisement calls (Green et al., 2019), making them a potential model for studying changes in vocal production in the context of age- and noise-induced hearing loss.

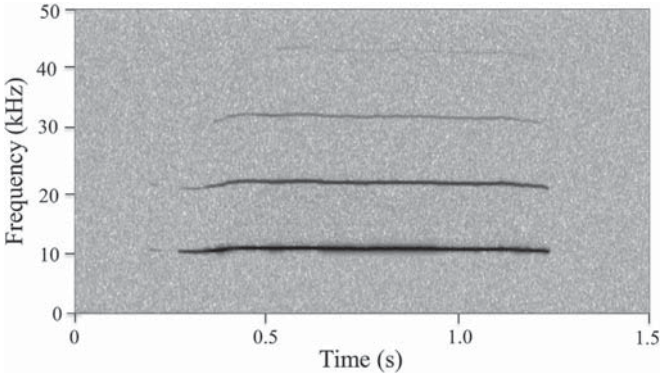

Fig. 1. Spectrogram of a long-distance advertisement vocalization of a northern grasshopper mouse.

In this study, we used a longitudinal design to measure auditory brainstem responses and vocalizations of control and noise-exposed grasshopper mice across the lifespan. We hypothesized that, similar to other rodents, grasshopper mice would lose hearing gradually, starting with high frequencies and progressing to lower frequencies. We predicted that noise-exposed mice would show decreased ABR sensitivity compared to controls after accounting for age. We also recorded vocalizations of mice at various ages before and after noise exposure to examine changes in voice. Although changes in voice during adulthood have not yet been measured in rodents, we hypothesized that peak fundamental frequency, duration, amplitude (dB SPL), and HNR of a call would change with age and noise-exposure as previously demonstrated in humans and other mammals.

2. Materials and methods

2.1. Subjects

Subjects used in the study were adult (80 – 1231 days old, d.o. hereafter) F1 and F2 offspring of wild-captured northern grasshopper mice (*Onychomys leucogaster*) near Animas, NM and Deadman Flat, 28 km north of Flagstaff, AZ. Animals were maintained on a 14:10 light:dark cycle and fed rodent chow and water ad libitum in animal facilities at Northern Arizona University, Flagstaff, AZ. All procedures were approved by the Northern Arizona University's Institutional Animal Care and Use Committee.

2.2. Auditory Brainstem Response (ABR)

ABRs were recorded to evaluate the status of the auditory nerve and brainstem response to sounds in control and noise-exposed mice of various ages. Baseline ABRs were collected from 14 female (F) and 12 male (M) mice. The mice were then randomly assigned

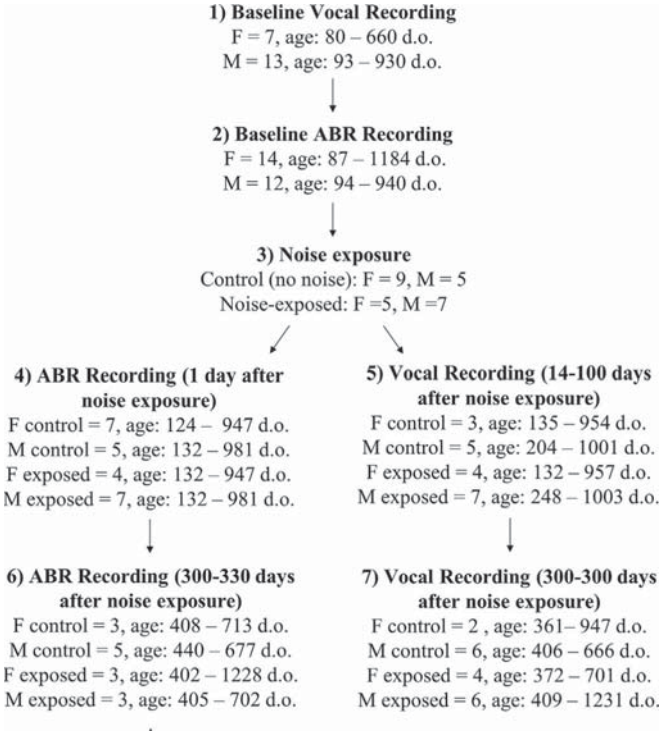

Fig. 2. Experimental design employed in this study. Steps are shown consecutively with all mice partaking in both vocal recording and ABR recording steps of this experiment.

to control (no noise exposure, F = 9, age: 87 – 1184 d.o.; M = 5, age: 94 – 940 d.o.) or noise-exposure conditions (F = 5, age: 94 – 910 d.o.; M = 7, age: 94 – 940 d.o.). In noise-exposed mice, ABRs were collected one day after noise exposure (F control = 7, age: 124 – 947 d.o.; M control = 5, age: 132 – 981 d.o.; F noise-exposed = 4, age: 132 – 947 d.o; M noise-exposed = 7, age: 132 – 981 d.o.), and again 300 – 330 days after baseline ABR recordings (F control = 3, age: 408 – 713 d.o.; M control = 5, age: 440 – 677 d.o.; F noise-exposed = 3, age: 402 – 1228 d.o.; M noise-exposed = 3, age: 405 – 702 d.o.) (Fig. 2). For a subset of animals, one day after noise exposure thresholds could not be calculated for some stimuli because the sensitivity was above the maximum amplitude that we presented in this experiment. This effect has been previously observed in laboratory mice and may occur due to permanent damage to hair cells, pre-existing hearing-health conditions, and genetic and biological factors (Amanipour et al., 2018; Davis et al., 2001; Guthrie and Xu, 2012; Guthrie, 2017; Kujawa and Liberman, 2009). Attrition due to age also occurred in the control group across the ABR recordings. Following measurement of mass, we administered ketamine/dexmedetomidine (75/0.5 mg/kg) intraperitoneally to anesthetize mice. Anesthetized animals were transferred to a 7″ x 15″ surgical table with built-in temperature control (VWR International LLC, Visalia, CA., USA) in a double-walled sound-isolation chamber (Industrial Acoustics Company, Inc., Bronx, NY).

Monaural (right ear) ABR measurements were obtained by placing three subdermal needle electrodes (VIASYS NeuroCare, Madison, WI) on the mastoid of right ear receiving the stimulus (active channel), the vertex (reference), and in the dorsum close to the base of the tail (ground). A transducer probe assembly was physically and acoustically coupled to the external auditory meatus of each mouse. Acoustic delays introduced by the transducer probe assembly were corrected for during each recording (Guthrie, 2016). During these neurophysiologic recordings, the transducer diaphragm was driven with alternating polarity.

2.2.1. Acoustic stimuli presentation

Intelligent Hearing Systems hardware and software (SmartEP Windows USB, version 3.94b, Intelligent Hearing Systems, Miami, FL) were used for presentation of calibrated stimuli, signal acquisition and manipulation, equipment control, and data management. Acoustic stimuli were digitally synthesized and consisted of 512 presentations of pure tones (1.56-ms Blackman envelope) and clicks (100-μs rectangular voltage pulse). In each trial, click stimuli (100–3000 Hz) were presented first, followed by pure tones in ascending order (4, 8, 12, 16, 24, and 32 kHz). All stimuli were presented at a rate of 19.3/s at decreasing amplitudes from 100 to 0 dB SPL in 5 dB steps. A Sound Booster Box with high pass filter was used to drive a high-frequency transducer (Intelligent Hearing Systems) for pure-tone stimulation of the right ear. The electroencephalographic responses to the pure tones were amplified (100,000 times), and bandpass filtered (Frequencies: 100–1500 Hz/ Clicks: 100–3000 Hz). Presentation of a high amplitude (e.g. 100–60 dB) stimulus was terminated when noise-exposed mice exhibited no ABR responses. All trials lasted approximately 50 min. After each trial, animals were placed on a flat, clean surface within their home cage over a heating pad and monitored until fully recovered.

2.3. Noise exposure

Mice (M = 7, age range: 94 – 940 d.o.; F = 5, age range: 94 - 910 d.o.) were exposed to noise after the baseline ABR measurements for 4 h at 105 dB SPL using a noise stimulus consisting of a 7000 Hz white-noise band (9–16 kHz) centered at 12.5 kHz. This noise exposure reliably produces permanent loss of function and dead hair cells in laboratory mice as measured by distortion product otoacoustic emissions, ABRs, whole-nerve compound action potentials, and cytocochleograms (Guthrie and Xu, 2012; Guthrie, 2017, 2011). The exposure paradigm has been described in detail previously (Guthrie et al., 2011; Guthrie, 2017). Briefly, awake and alert animals were placed in a 15 × 13 × 11 cm wire-cloth enclosure placed inside a reverberant 40 L chamber. To avoid audiogenic seizures, noise was initially broadcast at 60 dB SPL. Noise loudness was then increased in 5 dB steps every 5 min until 105 dB was attained. Animals were visually monitored for physical signs of stress (e.g., hyperactivity, excessive grooming) throughout the entire procedure. The noise was produced by a function generator (Stanford Research Systems Model DS335, Menlo Park, CA) coupled to a frequency device (Frequency Devices Model 9002, Haverhill, MA). An amplifier (Denon Model DRA-295) was used to deliver the noise via a speaker (ScanSpeak D2606/922000, Videbaek, Denmark) located approximately 5 cm above the wire-cloth enclosure. A sound pressure meter (OB-300 Quest Type-1) with 1/3 octave filter set (Quest Electronics, Oconomowoc, WI, USA- Weighting: lin; Response: slo; Mode: SPL; dB: 60–120; Time mode: 1/3) was used to verify the sound pressure level and noise frequency spectrum.

2.4. Acoustic recordings

Long-distance vocalizations were recorded from 20 mice (F = 7, M = 13) prior to collection of baseline ABRs, after noise exposure (F control = 3, age: 135 – 954 d.o.; M control = 5, age: 204 – 1001 d.o.; F noise-exposed = 4, age: 132 – 957 d.o.; M noise-exposed = 7, age: 248 – 1003 d.o.), and again 300 – 330 days after noise-exposure (F control = 2, age: 361 – 947 d.o.; M control = 6, age: 406 – 666 d.o.; F noise-exposed = 4, age: 372 – 701 d.o.; M noise-exposed = 6, age: 409 – 1231 d.o.) (Fig. 2). High frequency USVs were not recorded nor investigated in this study. Individually-housed animals in their home cage were placed within a semi-anechoic sound cubicle for overnight (10 h) recording for 3 nights, or until the mouse produced at least one call. If

a mouse did not produce a vocalization after 21 days, they were removed from the recording experiment, or re-recorded at a later date. We used a 1/4″ microphone (Type 40BE, G.R.A.S.) connected to preamplifiers (Type 26 CB, G.R.A.S.) to record spontaneous vocalizations. The microphone response was flat within ±1.5 dB from 10 Hz to 50 kHz, and the pre-amplifier response was flat within ± 0.2 dB from 2 Hz to 200 kHz. Microphones were connected to a National Instruments DAQ (USB 4431) sampling at 102.4 kHz to a laptop computer running a custom MATLAB (v. 2018a) script . Playback of a 13 kHz pure tone from different areas of the cage within the cubicle showed that recordings vary by ca. 1 dB.

2.5. Data analysis

2.5.1. ABR experiment and statistical analyses

We used the visual detection method (Chen et al., 2016; Gall et al., 2011; Jacobson, 1985) to determine the lowest stimulus level (dB) per stimulus that evoked an ABR response. Thresholds were operationally defined as the dB level halfway (2.5 dB) between the last detectable ABR response and next lowest stimulus level.

Given the longitudinal design of this study, our data set contained an unequal number of observations from every mouse per age category due to differences in lifespan and responses to noise-exposure. In other words, each condition contained a different number of observations per individual, with some mice contributing multiple points per stimulus, while others did not evoke ABRs following noise-exposure or due to ARHL. Thus, we developed an exploratory linear mixed effects model examining main effects of age, sex, stimulus, and noise-exposure condition (Supplementary Table 1). Overall, identity accounted for 37% of variability in our data. Then, exploratory regression analyses were performed to determine if aging variables (age, age^2, age^3) explained the increase in ABR thresholds in control and noise-exposed groups across the lifespan differently. Such polynomial variables have been previously used to model hearing loss in humans and in mice (Kobrina and Dent, 2019; Kobrina et al., 2020; Lin et al., 2011; Ohlemiller et al., 2010; Pearson et al., 1995). The polynomial variable has been linked to age-related cochlear degeneration as reflected by endocochlear potential decline, and loss of hair cells, strial marginal cells, outer sulcus cells, and loss of auditory nerve fibers in CBA/CaJ mice (Kobrina et al., 2020; Ohlemiller et al., 2010). The goodness of fit for different functions per noise-exposure condition and sex was assessed using a correlation coefficient comparison tool in R (*cocor.dep.groups.()* in the *cocor* R package) (Diedenhofen and Musch, 2015). In the control condition, both first (threshold (dB) = y0 + a*x) and second order functions (threshold (dB) = y0 + a*x + bx^2) explained the greatest amount of variability in the threshold data across stimuli for females (32 – 46%), and these functions were not significantly different (p = 0.109). For males, a second order polynomial function explained the greatest amount of variability in hearing across stimuli (45%). The second order polynomial was a better data fit than the first order polynomial (p = 0.032). For both sexes in the control condition, there were no significant differences between second and third order polynomials (p > 0.05). In the noise-exposed condition, a first order polynomial was the only function that predicted variability in threshold data for females (7%, p = 0.046). For males, second and third order polynomial functions served as significant predictors of variability in threshold data (6–8%, p > 0.05) and were not significantly different (p = 0.417). Thus, our final models incorporated both linear (age) and polynomial (age^2) variables for further analyses.

We used linear mixed-effects models to examine whether changes in age and age^2 predicted changes in ABRs between the sexes in control and noise-exposed groups separately (LMM, lmer in the lme4 R package; Bates et al., 2014; R Core Team, 2014). In the control group, we examined if thresholds (dB SPL) were affected by fixed factors of sex (female vs. male), stimulus (click, 4, 8, 12, 16, 24, and 32 kHz tones), and random factors of age and age^2, as well as by interactions between age, age^2, and sex. Planned post-hoc comparisons using Tukey's method were performed to assess the relationship between age, age^2, sex, and stimulus type in mice for three age categories (350, 700, and 1000 d.o.) using the *emmeans* package in R (Lenth, 2020). The age categories were pre-determined as ages of interest due to their significance in behavioral, anatomical, and electrophysiological changes in hearing in laboratory mice (Henry, 2004; Kobrina and Dent, 2019; Kobrina et al., 2020; Ohlemiller et al., 2010; Zheng et al., 1999). In the noise-exposed group, we examined if thresholds (dB SPL) were affected by fixed factors of sex (female vs. male), stimulus (click, 4, 8, 12, 16, 24, and 32 kHz tones), and random factors of age, age^2, and age at noise exposure, as well as by interactions between age, age^2 and sex. To control for dependencies from sampling each mouse repeatedly, we included a random intercept for mouse identity across age. Planned post-hoc comparisons using Tukey's method were performed to assess the relationship between age, sex, and stimulus type in mice before and after noise exposures (*emmeans* R package; Lenth, 2020). We then calculated the mean threshold shift across stimuli between baseline, one day after noise exposure, and 300 – 330 days later. Significance levels were adjusted to account for multiple comparisons based on the number of models (n = 3, p = 0.016).

2.5.2. Acoustic recording and analyses

Previous analyses of grasshopper mouse vocalizations indicate that calls are sexually monomorphic (Pasch et al., 2016, 2017; Green et al., 2019). Nevertheless, sex is an important factor in the progression of ARHL (Henry, 2004; Kobrina and Dent, 2019; Ohlemiller, 2018), thus we considered sex as a factor in age-related changes to the voice. Band-pass filtered (7–15 kHz) calls were detected using an automated algorithm in Avisoft SASLab Pro (version 4.2.27, Avisoft Bioacoustics, Germany; 256-point Fast Fourier Transform [FFT]; Hann window with 50% overlap; frequency resolution 750 Hz, temporal resolution 0.67 ms). We extracted peak F_0 (kHz), duration (s), call amplitude (dB SPL), and HNR. HNR is the parameter that quantifies noise in animal vocalizations or speech resulting from turbulent airflow due to inadequate vocal fold closure or aperiodic vocal fold vibration (Ferrand, 2002). HNR is calculated as a ratio of harmonic to nonharmonic noise floor. First, the nonharmonic noise floor of the spectrum is estimated by calculating the moving average of the spectrum. Then, the maximum distance between the original spectrum and the filtered spectrum is calculated as the HNR measure (Avisoft-SASLab Pro, v. 5.2). Sound pressure levels (dB SPL re: 20 μPa) of each vocalization were calculated in MATLAB (2018a) from calibrated microphones.

Similar to the ABR analyses, our call data set contained an unequal number of observations from every mouse per age category due to differences in calling rate, lifespan, and noise-exposure condition. Separate mixed-effects linear models were used to examine whether changes in age predicted changes in F_0, duration, call amplitude, and HNR in mice across stimuli (LMM, lmer in the lme4 R package; Bates et al., 2014; R Core Team, 2014). In these four models, we examined if the predictor variables were affected by fixed factors of sex (male vs. female), noise-exposure (control vs. noise-exposed), and random factors of age and mass, as well as by interactions between sex, condition, and age. To control for dependencies from sampling each mouse repeatedly, we included a random intercept for mouse identity across age. Post-hoc comparisons using Tukey's method were performed to assess the relationship between sex, condition, and age at three age categories (350, 700, and 1000 d.o.) (*emmeans* R package; Lenth, 2020). Significance

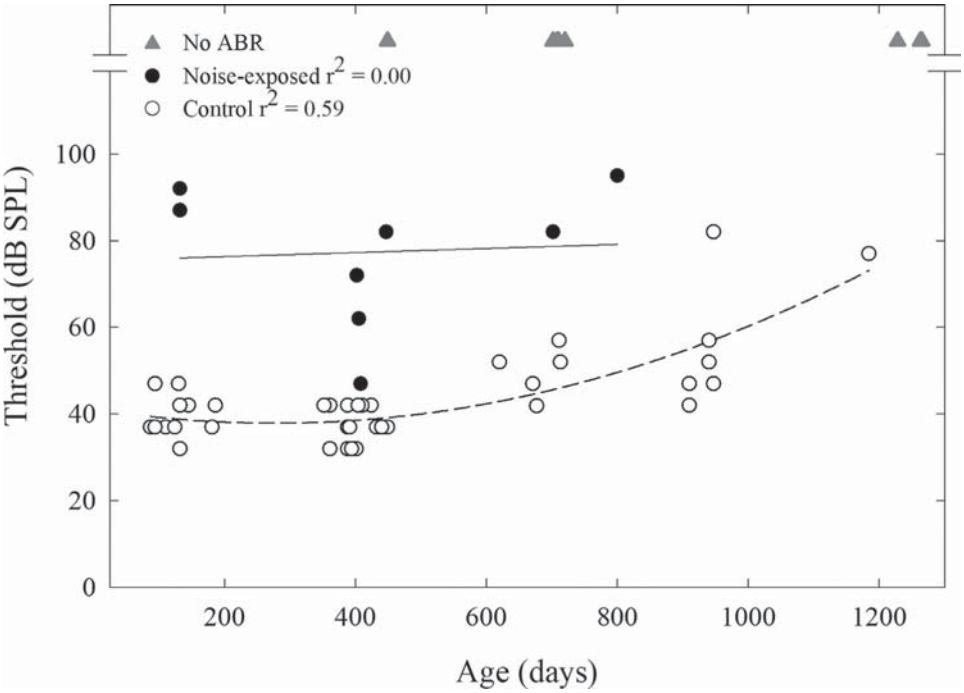

Fig. 3. Age- and noise-exposure related deterioration of the auditory brainstem response (ABR) for the click stimulus collapsed across sex in grasshopper mice. This plot depicts ABR thresholds from multiple mice across their lifespans (control condition = open circles (baseline (pre noise exposure): $N = 12$, control: $N = 14$, age: 94–1184 d.o.), noise-exposed = filled circles ($N = 6$, age: 132–800 d.o.). Note, 12 noise-exposed mice were tested, only 4 of them contributed data one day after noise exposure, and 4 contributed data 300–330 days after noise exposure. Black lines represent the best data fit for control (dashed) and noise-exposed mice (solid). The amount of variability in data explained by aging for each exposure condition is expressed in the form of r^2 for control (C) and noise-exposed (NE) mice. Mice that lacked ABR responses for the click stimulus post-exposure are represented by gray triangles ($N = 8$, age: 447–1265 d.o.).

levels were adjusted to account for multiple comparisons based on the number of models ($n = 4$, $p = 0.0125$).

Lastly, we examined whether changes in hearing sensitivity due to age-related and noise-induced hearing loss were correlated with changes in call properties. We used Spearman's correlation to assess these relationships due to the non-normal distribution of data.

3. Results

Aging explained changes in ABR thresholds for all stimulus frequencies in the control condition (collapsed across sex, $p < 0.002$). Second order polynomial functions explained the greatest amount of variability in ABR data across stimuli in the control mice (linear: 14 – 46% vs. polynomial: 22 – 59%). Aging did not account for changes in hearing sensitivity in noise-exposed mice ($p > 0.083$). Linear and polynomial functions explained similar amounts of variability in noise exposed data (0 – 21%) (Figs. 3 and 4).

3.1. ABR results for control group

Age, age^2, sex, and stimulus type were significant predictors of the increase in ABR thresholds in grasshopper mice (Table 1). In addition, we found a significant age * sex interaction. In general, mice exhibited peak sensitivity (i.e. lowest ABR thresholds) in the 8 – 24 kHz region of the audiograms, with higher thresholds for other stimuli (Fig. 5). Female mice had significantly higher thresholds for a click stimulus than for 8 ($p < 0.001$), 12 ($p < 0.001$), 16 ($p < 0.002$), and 24 kHz ($p = 0.002$). In addition, females had less sensitive hearing for 4 kHz than for 24 kHz ($p = 0.021$), and for 32 kHz than for 8 ($p = 0.014$), 12 ($p = 0.021$), and 24 kHz tones ($p < 0.001$). Males showed a similar pattern, with significantly higher thresholds for a click stimulus than for 8 ($p < 0.001$), 12 ($p < 0.001$), 16 ($p < 0.002$), and 24 kHz ($p = 0.002$). In addition, male mice exhibited higher thresholds for 32 kHz than for 8 ($p = 0.014$), 12 ($p = 0.021$), and 24 kHz tones ($p < 0.001$). Male mice experienced a 53.4 dB threshold shift due to aging across all stimuli, while females experienced an average of 24.6 dB threshold shift (Fig. 5). Female mice retained normal hearing abilities across stimuli into middle adulthood (700 d.o., $p = 0.017$), losing hearing between 700 and 1000 d.o. ($p < 0.001$). In contrast, male mice lost hearing progressively across the lifespan ($p < 0.001$; Fig. 5).

Table 1
Mixed-effects model analysis and significance testing comparing the ABR changes due to age in female ($N = 14$, $N_{observations} = 111$) and male ($N = 12$, $N_{observations} = 119$) grasshopper mice in the control group across the lifespan for click stimulus and six pure tone pips.[a].

Fixed Effects	*B*	*SE*	*t-value*	*p*
Intercept (Id)	42.52	2.66	15.95	**<0.001**
Age	82.39	22.73	3.62	**0.001**
Age^2	107.86	21.65	4.98	**<0.001**
Sex	6.27	3.36	1.87	0.067
4 kHz	−6.59	2.25	−2.94	**0.004**
8 kHz	−12.20	2.25	−5.43	**<0.001**
12 kHz	−11.90	2.25	−5.30	**<0.001**
16 kHz	−9.01	2.25	−4.02	**<0.001**
24 kHz	−13.87	2.25	−6.18	**<0.001**
32 kHz	−4.63	2.25	−2.06	**0.041**
Age * Sex	142.00	51.67	2.75	**0.007**
Age^2 * Sex	68.60	48.61	1.41	0.160
Random Effects	σ^2			
Mouse Id	6.43			
Residual	9.05			

[a] Significant values are bolded. LMM formula in R was lmer (threshold ~ sex * poly (age, 2) + stimulus + (1| Id)). *B* = model estimate, *SE* = standard error, σ^2 = standard deviation. Fixed effects for 4, 8, 12, 16, 24, and 32 kHz stimuli are compared to the click stimulus. Fixed effects for the age * sex interaction are compared to age * female interaction.

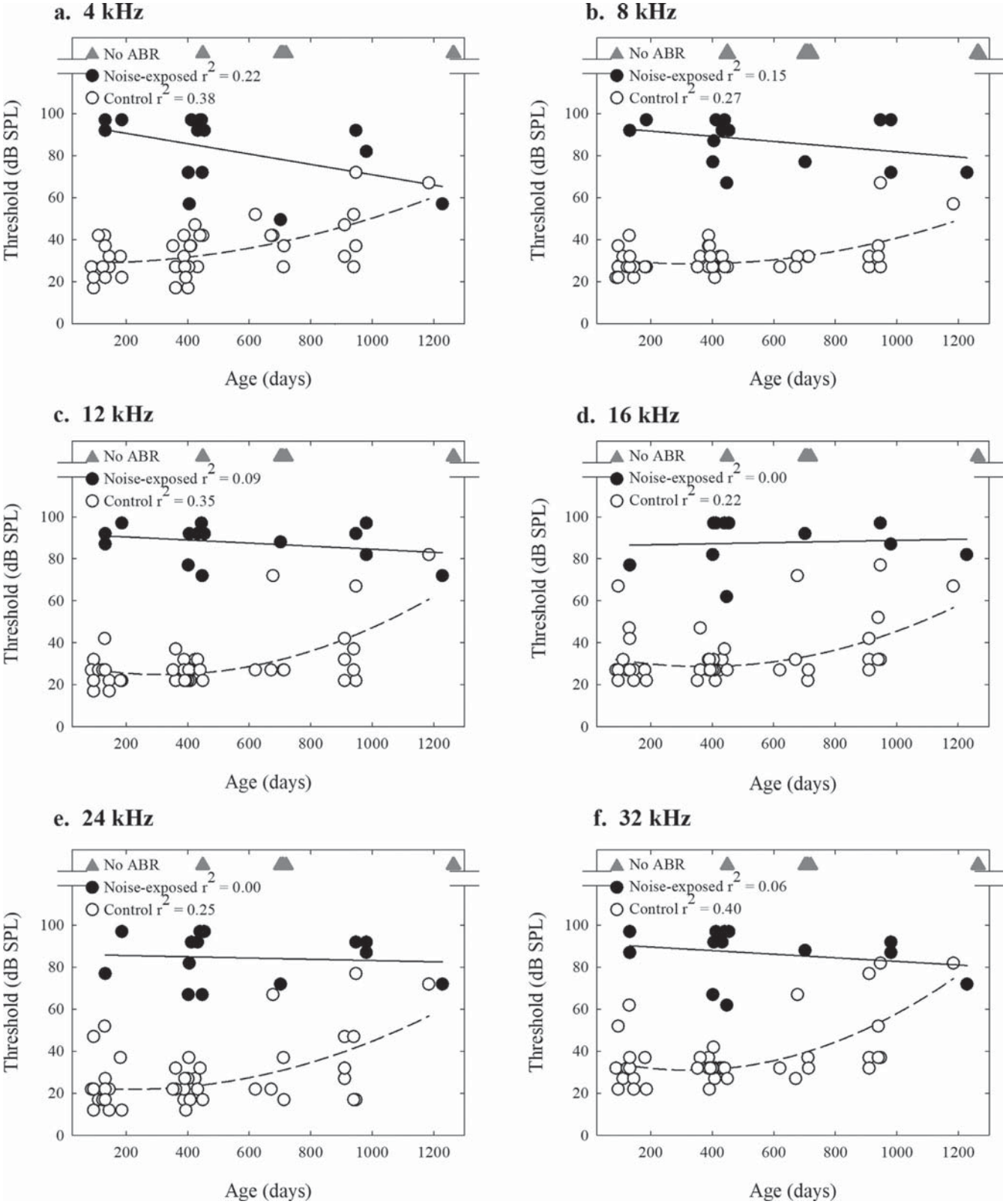

Fig. 4. Age- and noise-exposure related deterioration of the auditory brainstem response (ABR) for 4, 8, 12, 16, 24, and 32 kHz stimuli collapsed across sex (baseline pre noise exposure: $N = 12$, control: $N = 14$, age: 94 – 1184 d.o.; noise-exposed $N = 11$, age: 132 – 1228 d.o.) (a–f) in grasshopper mice. Note, 12 noise-exposed mice were tested, only 11 mice contributed data one day after noise exposure, and 6 mice contributed data 300 – 330 days after noise exposure These plots depict ABR thresholds from multiple mice across their lifespans (control condition = open circles, noise-exposed = filled circles). Black lines represent the best data fit for control (dashed) and noise-exposed mice (solid). The amount of variability in data explained by aging for each exposure condition is expressed in the form of r^2 for control (C) and noise-exposed (NE) mice. Mice that lacked ABR responses for tones post-exposure are represented by grey triangles ($N = 6$, age: 447 – 1265 d.o.).

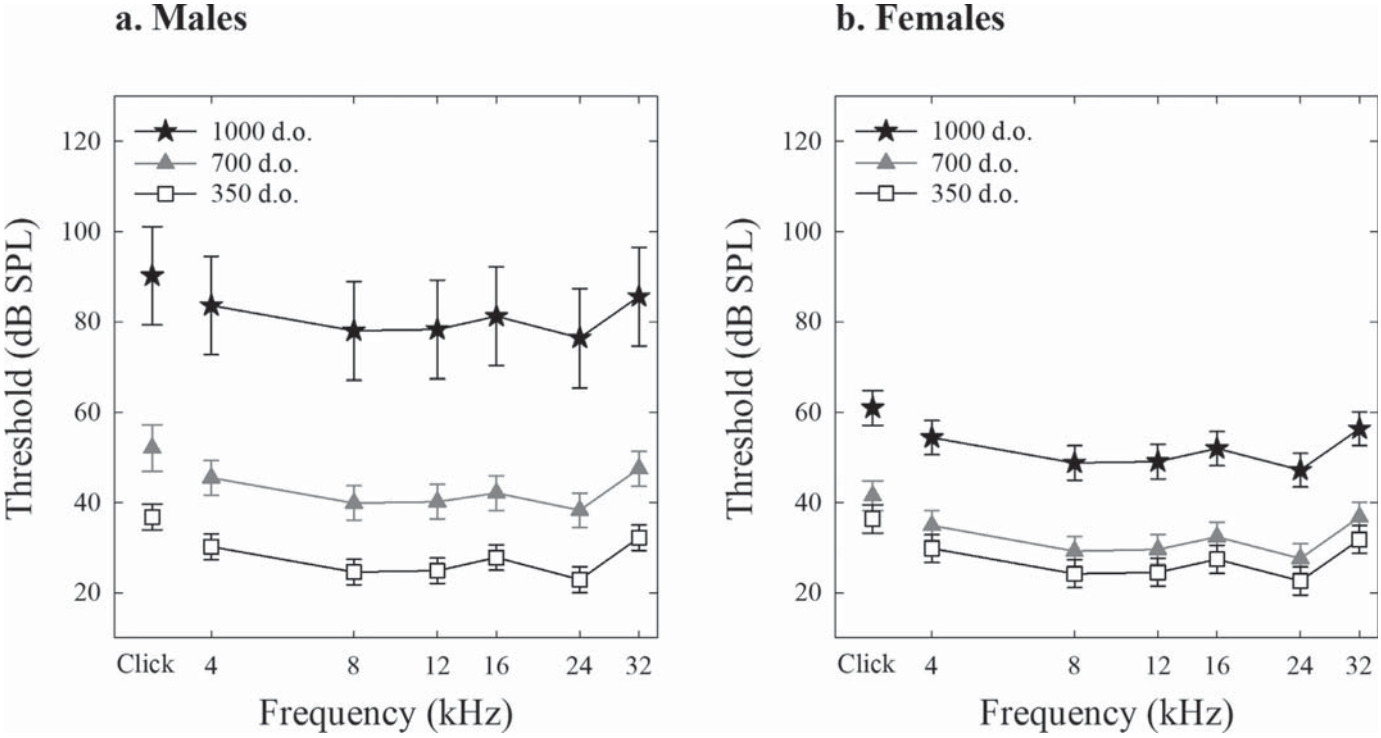

Fig. 5. Estimated marginal mean audiograms for male (a) and female (b) grasshopper mice in the control condition at 350 d.o. (350 = white squares), 351–700 d.o (700 = gray circles), and 701–1000 d.o (1000 = black stars) with standard error bars.

Table 2
Mixed-effects model analysis and significance testing comparing the ABR changes due to age in female (N = 5, $N_{observations}$ = 58) and male (N = 8, $N_{observations}$ = 103) grasshopper mice in the noise-exposed group across the lifespan for click stimulus and six pure tone pips.[a].

Fixed Effects	*B*	*SE*	*t-value*	*p*
Intercept (Id)	86.20	12.73	6.77	***<0.001***
Age	428.28	96.71	4.43	***<0.001***
Age^2	−50.23	42.51	−1.18	*0.240*
Sex	8.35	7.52	1.10	*0.337*
4 kHz	5.92	8.61	0.69	*0.493*
8 kHz	11.31	8.54	1.32	*0.188*
12 kHz	8.63	8.60	1.00	*0.317*
16 kHz	6.03	8.87	0.68	*0.498*
24 kHz	3.49	8.70	0.40	*0.689*
32 kHz	7.42	8.76	0.85	*0.399*
Age at noise exposure	−0.09	0.02	−3.72	***0.001***
Age * Sex	−11.71	88.85	−0.13	*0.900*
Age^2 * Sex	55.00	77.50	0.71	*0.484*
Random Effects	σ^2			
Mouse Id	9.21			
Residual	26.91			

[a] Significant values are bolded. The LMM formula in R was lmer (threshold ~ sex * age + stimulus + age at noise exposure + (1| Id)). *B* = model estimate, *SE* = standard error, σ^2 = standard deviation.

For both sexes, the greatest decrease in sensitivity occurred after 700 d o. (threshold increase by 19.5 dB for females and 38.1 dB for males, $p < 0.001$), although males had significantly higher ABR thresholds than females by 1000 d.o. ($p = 0.011$).

3.2. ABR results for noise-exposed group

Age and age at noise exposure were the only significant predictors of the increase in ABR thresholds in noise-exposed grasshopper mice (Table 2). There were no sex or stimulus differences after noise exposure, therefore all post-hoc analyses were combined across sex and stimulus type. Mice of all ages experienced a 61.55 dB threshold increase after noise exposure ($p < 0.001$), and a 21.25 dB recovery 300 – 330 days after the noise exposure ($p < 0.001$). Eight out of 12 mice did not recover after noise exposure and no ABRs could be collected from these subjects 300 – 330 days after noise exposure, however the age at which these subjects were noise-exposed was not related to recovery ($p = 0.451$). Furthermore, post hoc analysis revealed no significant differences between young and old male and female mice that were noise-exposed ($p > 0.05$).

3.3. Call recording results

We obtained acoustic recordings from 10 individual control (3 F – 161 calls, age: 80 – 954 d.o.; 7 M – 107 calls, age: 93 – 1001 d.o.) and 11 noise-exposed mice (4 F – 349 calls, age: 83 – 957 d.o.; 7 M – 288 calls, age: 83 – 1231 d.o.). Baseline measures for all acoustic variables did not differ between control and noise-exposed groups (all $p > 0.0125$). Age and mass were significant predictors of call F_0 (Table 3). In addition, condition * age and age * sex interactions were significant predictors of changes in F_0 (Table 3). Post-hoc comparisons indicated that mass was not related to F_0 across conditions ($t = 2.77$, $p = 0.029$). In general, F_0 decreased with age in control female (350 d.o. $M = 10.49$ dB, $SE = 0.52$; 700 d.o. $M = 9.78$ dB, $SE = 0.53$; 1000 d.o. $M = 9.18$ dB, $SE = 0.58$; t = 4.00, $p < 0.001$) and male mice across the lifespan (350 d.o. $M = 9.86$ dB, $SE = 0.29$; 700 d.o. $M = 8.51$ dB, $SE = 0.34$; 1000 d.o. $M = 7.35$ dB, $SE = 0.45$; t = 6.66, $p < 0.001$). Female mice had significantly higher F_0 at 1000 d.o. than males ($t = 2.59$, $p = 0.012$). Male mice in the control condition produced significantly lower F_0 calls than noise-exposed males at 350 (control: $M = 9.86$ dB, $SE = 0.29$; noise-exposed: $M = 10.84$ dB, $SE = 0.30$; $t = -3.08$, $p = 0.003$), 700 (control: $M = 8.51$ dB, $SE = 0.34$; noise-exposed: $M = 10.67$ dB, $SE = 0.48$; $t = -6.36$, $p < 0.001$), and 1000 d.o. (control: $M = 7.35$ dB, $SE = 0.45$; noise-exposed: $M = 10.52$ dB, $SE = 0.38$; $t = -6.98$, $p < 0.001$). Mice of both sexes in the noise-

Table 3
Mixed-effects model analysis and significance testing comparing changes in the peak frequency (kHz) of a grasshopper mouse call due to age and noise-exposure condition in female (N = 7, $N_{observations}$ = 510) and male (N = 13, $N_{observations}$ = 394) mice across the lifespan.[a].

Fixed Effects	*B*	*SE*	*t-value*	*p*
Intercept (Id)	$1.01 * 10^{1}$	$5.97 * 10^{-1}$	16.93	***<0.001***
Age	$-2.00 * 10^{-3}$	$4.95 * 10^{-4}$	−4.05	***<0.001***
Condition	$-7.78 * 10^{-1}$	$6.63 * 10^{-1}$	−1.17	*0.252*
Sex	$2.18 * 10^{-2}$	$6.23 * 10^{-1}$	0.04	*0.972*
Mass	$2.87 * 10^{-2}$	$1.02 * 10^{-2}$	2.81	***0.005***
Condition * Age	$1.76 * 10^{-3}$	$5.64 * 10^{-4}$	3.12	***0.002***
Age * Sex	$-1.86 * 10^{-3}$	$6.99 * 10^{-4}$	−2.66	***0.008***
Condition * Sex	$5.82 * 10^{-1}$	$7.82 * 10^{-1}$	0.74	*0.461*
Condition * Age * Sex	$1.61 * 10^{-3}$	$8.65 * 10^{-4}$	1.86	*0.064*
Random Effects	σ^2			
Mouse Id	0.80			
Residual	0.58			

[a] Significant values are bolded. The LMM formula in R was lmer (peak frequency ~ age * noise-exposure condition (i.e. condition) * sex + mass + + (1| Id)). *B* = model estimate, *SE* = standard error, σ^2 = standard deviation. Fixed effects for the condition * age are compared to the control condition * age interaction. Fixed effects for the age * sex interaction are compared to age * female.

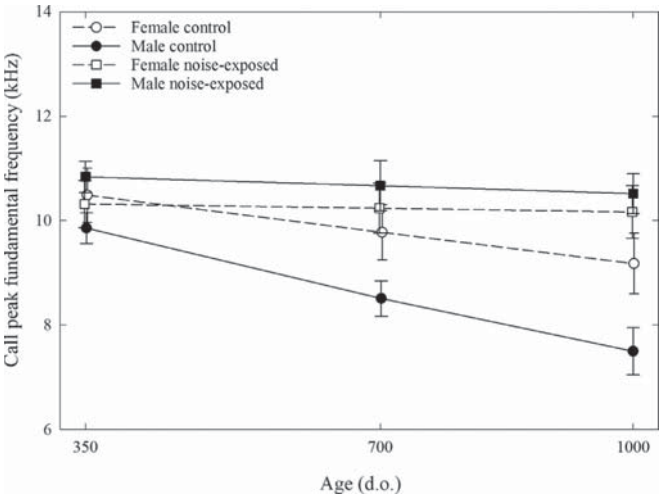

Fig. 6. Estimated marginal means for age-related changes of the peak fundamental frequency (kHz) of a grasshopper mouse's advertisement call with standard error bars in female (open circles and squares) and male (black circles and squares) mice, in control (circles) and noise-exposed conditions (squares) for three ages of interest (350, 700, 1000 d.o.).

exposed group did not experience changes in F_0 across the lifespan ($p > 0.05$) (Fig. 6).

Age, sex, and mass were significant predictors of call duration. In addition, condition * age and age * sex interactions were significant predictors of changes in call duration (Table 4). Mass was a significant predictor of call duration with mice of lower mass producing longer calls ($t = -5.43$, $p < 0.001$). In general, female mice in the control (350 d.o. M = 0.72 s, SE = 0.12; 700 d.o. M = 0.89 s, SE = 0.12; 1000 d.o. M = 1.03 s, SE = 0.13; t = - 4.43, $p < 0.001$) and the noise-exposed groups (350 d.o. M = 0.92 s, SE = 0.07; 700 d.o. M = 0.99 s, SE = 0.07; 1000 d.o. M = 1.04 s, SE = 0.09; t = - 2.96, p = 0.009) exhibited an increase in call duration with age, whereas males in both groups showed no changes ($p > 0.05$) (Fig. 7). There were no significant differences between sexes across conditions ($p > 0.05$). The condition and condition * sex interaction were significant predictors of HNR of a call (Table 5). However, post-hoc tests indicated no differences between noise-exposed and control groups for HNR across the lifespan (control: M = 23.6, SE = 0.18, noise-exposed: M = 23.4, SE = 0.13;

Table 4
Mixed-effects model analysis and significance testing comparing changes in the duration of a grasshopper mouse call due to age and noise-exposure condition in female (N = 7, $N_{observations}$ = 510) and male (N = 13, $N_{observations}$ = 394) mice across the lifespan.[a].

Fixed Effects	*B*	*SE*	*t-value*	*p*
Intercept (Id)	1.01	$1.32 * 10^{-1}$	7.68	***<0.001***
Age	$4.80 * 10^{-4}$	$1.07 * 10^{-4}$	4.49	***<0.001***
Condition	$1.09 * 10^{-1}$	$1.47 * 10^{-1}$	0.74	*0.466*
Sex	$3.09 * 10^{-1}$	$1.38 * 10^{-1}$	2.24	***0.032***
Mass	$-1.22 * 10^{-2}$	$2.21 * 10^{-3}$	−5.50	***<0.001***
Condition * Age	$-2.87 * 10^{-4}$	$1.22 * 10^{-4}$	−2.35	***0.019***
Age * Sex	$-2.97 * 10^{-4}$	$1.51 * 10^{-4}$	−1.96	***0.050***
Condition * Sex	$7.51 * 10^{-3}$	$1.73 * 10^{-1}$	0.04	*0.966*
Condition * Age * Sex	$1.60 * 10^{-4}$	$1.87 * 10^{-4}$	0.86	*0.392*
Random Effects	σ^2			
Mouse Id	0.18			
Residual	0.13			

[a] Significant values are bolded. The LMM formula in R was lmer (duration ~ age * noise-exposure condition (i.e. condition) * sex + mass + session* noise-exposure condition (i.e. condition) + (1| Id)). *B* = model estimate, *SE* = standard error, σ^2 = standard deviation. Fixed effects for sex are compared to female. Fixed effects for the noise-exposure condition * age interaction are compared to control condition * age. Fixed effects for age * sex interaction are compared to age * female.

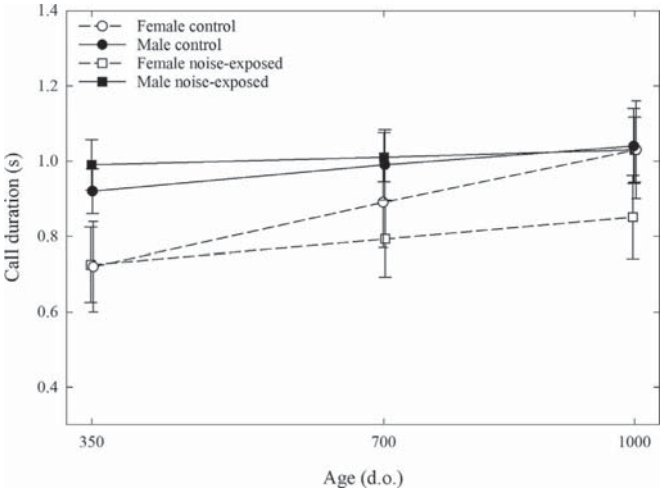

Fig. 7. Estimated marginal means for age-related changes of the duration (s) of a grasshopper mouse's advertisement call with standard error bars in female (open circles and squares) and male (black circles and squares) mice in control (circles) and noise-exposed conditions (squares) for three ages of interest (350, 700, 1000 d.o.).

Table 5
Mixed-effects model analysis and significance testing comparing changes in the harmonic-to-noise ratio (HNR) of a grasshopper mouse call due to age and noise-exposure condition in female (N = 7, $N_{observations}$ = 510) and male (N = 13, $N_{observations}$ = 394) mice across the lifespan.[a].

Fixed Effects	*B*	*SE*	*t-value*	*p*
Intercept (Id)	24.22	$3.45 * 10^{-1}$	70.23	***<0.001***
Age	$6.80 * 10^{-5}$	$4.72 * 10^{-4}$	0.14	*0.8863*
Condition	$-6.39 * 10^{-1}$	$2.83 * 10^{-1}$	−2.26	***0.039***
Sex	$-2.34 * 10^{-1}$	$3.07 * 10^{-1}$	−0.76	*0.457*
Mass	$-1.69 * 10^{-2}$	$1.05 * 10^{-2}$	−1.61	*0.110*
Condition * Age	$3.04 * 10^{-4}$	$5.66 * 10^{-4}$	0.54	*0.594*
Age * Sex	$2.26 * 10^{-4}$	$6.42 * 10^{-4}$	0.35	*0.726*
Condition * Sex	$8.54 * 10^{-1}$	$3.94 * 10^{-1}$	2.17	***0.043***
Condition * Age * Sex	$-6.69 * 10^{-4}$	$8.10 * 10^{-4}$	−0.83	*0.413*
Random Effects	σ^2			
Mouse Id	0.19			
Residual	0.76			

[a] The LMM formula in R was lmer (HNR ~ age * noise-exposure condition (i.e. condition) * sex + (1| Id)). *B* = model estimate, *SE* = standard error, σ^2 = standard deviation. Fixed effects for the noise-exposure condition are compared to control condition. Fixed effects for the noise-exposure condition * sex interaction are compared to control condition * female.

Table 6
Mixed-effects model analysis and significance testing comparing changes in the sound pressure level (SPL) of a grasshopper mouse call due to age and noise-exposure condition in female (N = 7, $N_{observations}$ = 510) and male (N = 13, $N_{observations}$ = 394) mice across the lifespan.[a].

Fixed Effects	*B*	*SE*	*t-value*	*P*
Intercept (Id)	$9.33 * 10^{1}$	1.65	56.78	***<0.000***
Age	$-2.21 * 10^{-3}$	$1.98 * 10^{-4}$	−1.05	*0.298*
Condition	−2.55	2.41	−1.81	*0.081*
Sex	−1.79	1.51	−1.19	*0.243*
Mass	$-6.68 * 10^{-2}$	$4.53 * 10^{-2}$	−1.47	*0.142*
Condition * Age	$3.69 * 10^{-3}$	$2.49 * 10^{-3}$	1.48	*0.141*
Age * Sex	$-5.25 * 10^{-5}$	$2.89 * 10^{-3}$	−0.02	*0.986*
Condition * Sex	3.11	1.92	1.62	*0.114*
Condition * Age * Sex	$-7.90 * 10^{-4}$	$3.62 * 10^{-3}$	−0.21	*0.827*
Random Effects	σ^2			
Mouse Id	1.16			
Residual	2.99			

[a] The LMM formula in R was lmer (SPL ~ age * noise-exposure condition (i.e. condition) * sex + mass + (1| Id)). *B* = model estimate, *SE* = standard error, σ^2 = standard deviation.

t = 1.14, p = 0.26). Lastly, there were no significant predictors of change in SPL (Table 6).

Finally, to further explore relationships between hearing and voice, we examined if the magnitude of hearing loss due to aging and noise-exposure across all stimuli was correlated to changes in F_0 and duration of grasshopper mouse calls. These analyses were performed only on call qualities that changed significantly with age (Tables 3 and 4). In control animals, we found that hearing loss was correlated with an increase in F_0 in females (r = 0.21, p = 0.03), but not in males (r = 0.05, p = 0.65). Hearing loss was not correlated with changes in duration in female (r = −0.06, p = 0.52) and male (r = −0.07, p = 0.47) mice. In noise-exposed animals, F_0 decrease correlated with hearing loss in males (r = −0.57, p < 0.001). However, F_0 was not correlated with hearing loss in noise-exposed females (r = −0.25, p = 0.06). Lastly, duration increase was correlated with hearing loss in noise-exposed males (r = 0.35, p < 0.001), but not in females (r = 0.01, p = 0.92).

4. Discussion

The goal of this experiment was to assess the association between ARHL, NIHL, and vocal production in grasshopper mice. We found that mice exhibited age- and noise-induced hearing loss that correlated with changes in vocal production. Similar to humans and other rodents, grasshopper mice progressively lost hearing across the lifespan, with hearing loss occurring more rapidly in males than females. Mice also experienced severe hearing loss across all frequencies after exposure to noise. Lastly, we found that the frequency and duration of advertisement calls were affected by hearing loss similar to findings in other mammals. We discuss our results in relation to modeling human hearing and voice disorders.

Hearing sensitivity in young grasshopper mice was consistent with previously established measures of hearing in this species (Green et al., 2019; Heffner and Heffner, 1985). However, mice experienced progressive ARHL and showed sexual dimorphism in hearing loss rates across the lifespan similar to humans (Gates and Cooper, 1991; Tambs et al., 2003) and various laboratory mouse strains (Henry, 2004; Kobrina and Dent, 2019; Zheng et al., 1999). Accelerated hearing loss in the last third of grasshopper mouse's lifespan (Fig. 3 and 4) is consistent with cochlear degeneration in laboratory mice (Kobrina et al., 2020; Ohlemiller et al., 2010). The degree of ARHL in grasshopper mice (25 – 53 dB) falls into the mild to moderate hearing loss categories for humans (Homan et al., 2017; Huang and Tang, 2010), although human hearing is commonly assessed using behavioral rather than electrophysiological techniques. Audiograms obtained using ABRs are often less sensitive than those obtained using behavioral methods (Radziwon et al., 2009). In addition, age-related changes in ABRs occur earlier than measurable behavioral changes in laboratory mice (Kobrina et al., 2020). Thus, our results provide a conservative estimate of hearing abilities and ARHL onset.

Rates of hearing loss differed between sexes after one year of life. While males showed early onset of ARHL and progressive hearing loss across frequencies, females exhibited a more abrupt hearing loss onset similar to humans (Gates and Cooper, 1991; Tambs et al., 2003). Consequently, males had lower hearing sensitivity than females after 350 d.o., similar to findings in laboratory mice (Henry, 2004; Kobrina and Dent, 2019; Kobrina et al., 2020). We did not observe severe hearing loss in females, suggesting that females may be a unique model for studying protective effects of feminization on the aging auditory system (Hultcrantz et al., 2006).

In addition to ARHL, grasshopper mice were highly susceptible to NIHL. Mice of all ages experienced a deterioration in hearing abilities one day after noise exposure. Similar to the CBA/CaJ mice, grasshopper mice experience elevated temporary threshold shifts shortly after noise exposure and improved sensitivity later in life (Amanipour et al., 2018). We found no relationship between age at noise-exposure and hearing recovery, although low sample size resulting from high attrition rates dampen our strength of inference. Alternative explanations exist to explain why mice may recover their hearing abilities. First, rodents may have an over-representation of outer hair cells sensitive to lower frequencies in their cochleae that enables compensation after noise exposure (Campo et al., 2003). Second, unique genetic backgrounds may act as protective factors in mice of various ages resulting in less detrimental effects after noise exposure (Davis et al., 2001; Hulterantz and Li, 1993; Lin et al., 2009). In laboratory mice, susceptibility to permanent hearing loss is due to the interaction between genetic factors associated with ARHL and NIHL. Specifically, mouse strains that express two recessive Ahl genes associated with hearing loss (Ahl/Ahl) were more susceptible to noise trauma than mouse strains with only one (+/Ahl) or no Ahl alleles (+/+; Davis et al., 2001). In addition, a variety of gene clusters associated with NIHL susceptibility and resistance have recently been identified (Myint et al., 2016). Although the genetic background of grasshopper mouse hearing is presently unknown, further investigation will help clarify the role of genes as hearing protective factors (Christie and Eberl, 2014; Hilliard et al., 2012).

Grasshopper mouse vocalizations exhibited diverse and variable responses to aging and noise exposure, sometimes in a sex-specific manner. Such variation is typical in studies of human vocal aging (Coelho et al., 2015) and in the absence of auditory feedback (Higgins et al., 2003, 2005). While some acoustic characteristics remained static (e.g. call amplitude and HNR), others exhibited complex patterns of change. In particular, call F_0 progressively decreased with age in control mice, with older females producing calls with higher F_0 than males. A similar decrease in F_0 was found in adult male rat 50 kHz USVs, although the mechanisms mediating sex differences are unclear (Basken et al., 2012; Peterson et al., 2013). Because female mice experienced a lesser degree of ARHL than males, which was correlated with changes in F_0, we speculate that estrogen may play an important role in auditory-vocal interactions. For example, decreased estrogen in menopausal women results in increased hearing loss (Hederstierna et al., 2010) and lower F_0 via mucosal edema of vocal folds (Emerich et al., 1996). In contrast, F_0 of noise-exposed mice did not exhibit an age-related decrease but instead remained high, similar to findings in hearing-compromised humans (Binnie et al., 1982; Mora et al., 2012), cats (Shipley et al., 1988), and laboratory mice (Arriaga et al., 2012). Control and noise-exposed females also produced longer calls in agreement with findings in deafened cats

(Hubka et al., 2015). Together our findings suggest that age- and noise-induced hearing loss contribute to the observed changes in vocal production. The physiological mechanisms underlying changes in both F_0 and duration may be due to changes in subglottal pressure, oral pressure, vocal fold composition and biomechanics, decreased motor control, and/or respiratory function (Higgins et al., 2003, 2005; Johnson et al., 2015; Perkell et al., 2000; Riede et al., 2017; Shipley et al., 1988). Disentangling the role of aging, use, hormones, and loss of auditory feedback on such diverse mechanisms will require further experimentation that considers interactions among mechanisms over time.

In conclusion, our data indicate that grasshopper mice exhibited age- and noise-induced hearing deficits that correlate with changes in call production. Grasshopper mice develop sexually dimorphic ARHL patterns, with male mice losing hearing progressively and having worse sensitivity than females. Some grasshopper mice were able to partially recover hearing abilities one year after noise exposure, although further research is necessary to understand the factors contributing to hearing loss and recovery. Grasshopper mice under natural and noise-exposed aging conditions produced calls differing in F_0 and duration, suggesting that hearing loss and other physiological processes may be responsible for vocal control in this species. Our findings highlight the importance of further study of hearing and vocal production mechanisms in a unique rodent model to better understand hearing and communication disorders.

CRediT authorship contribution statement

Anastasiya Kobrina: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Mahendra Kumar Hidau:** Conceptualization, Methodology, Investigation, Project administration. **Tobias Riede:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **O'neil W. Guthrie:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Bret Pasch:** Conceptualization, Methodology, Investigation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.heares.2021.108210.

References

Amanipour, R.M., Zhu, X., Duvey, G., Celanire, S., Walton, J.P., Frisina, R.D., 2018. Noise-induced hearing loss in mice: effects of high and low levels of noise trauma in CBA mice. In: Proceedings of the 2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). IEEE, pp. 1210–1213.

Arriaga, G., Jarvis, E.D., 2013. Mouse vocal communication system: are ultrasounds learned or innate? Brain Lang. 124 (1), 96–116.

Arriaga, G., Zhou, E.P., Jarvis, E.D., 2012. Of mice, birds, and men: the mouse ultrasonic song system has some features similar to humans and song-learning birds. PLoS One 7 (10), e46610.

Bailey, V., Sperry, C., 1929. Life history and habits of grasshopper mice. In: Genus Onychomys, 145. US Department of Agriculture, pp. 1–19 Tech Bull.

Baken, R.J., 2005. The aged voice: a new hypothesis. J. Voice 19 (3), 317–325.

Basken, J.N., Connor, N.P., Ciucci, M.R, 2012. Effect of aging on ultrasonic vocalizations and laryngeal sensorimotor neurons in rats. Exp. Brain Res. 219 (3), 351–361.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using lme4. J. Stat. Softw. arXiv:1406.5823.

Benjamin, B.J., 1982. Phonological performance in gerontological speech. J. Psycholinguist Res. 11 (2), 159–167.

Binnie, C.A., Daniloff, R.G., Buckingham Jr, H.W., 1982. Phonetic disintegration in a five-year-old following sudden hearing loss. J. Speech Hear. Disord. 47 (2), 181–189.

Blamey, P.J., Sarant, J.Z., Paatsch, L.E., Barry, J.G., Bow, C.P., Wales, R.J., Tooher, R., 2001. Relationships among speech perception, production, language, hearing loss, and age in children with impaired hearing. J. Speech Lang. Hear. Res. 44, 264–285.

Brainard, M.S., Doupe, A.J., 2000. Auditory feedback in learning and maintenance of vocal behaviour. Nat. Rev. Neurosci. 1 (1), 31–40.

Campo, P., Pouyatos, B., Lataye, R., Morel, G., 2003. Is the aged rat ear more susceptible to noise or styrene damage than the young ear? Noise Health 5 (19), 1–18.

Chen, J., Jono, T., Cui, J., Yue, X., Tang, Y., 2016. The acoustic properties of low intensity vocalizations match hearing sensitivity in the webber-toed gecko, *Gekko subpalmatus*. PLoS One 11, e0146677.

Christie, K.W., Eberl, D.F., 2014. Noise-induced hearing loss: new animal models. Curr. Opin. Otolaryngol. Head Neck Surg. 22 (5), 374–383.

Coelho, A.C., Medved, D.M., Brasolotto, A.G., 2015. Hearing loss and the voice. In: Bahmad, Jr., F. (Ed.), Update On Hearing Loss. IntechOpen, pp. 103–128. doi:10.5772/61217.

Davis, R.R., Newlander, J.K., Ling, X.B., Cortopassi, G.A., Krieg, E.F., Erway, L.C., 2001. Genetic basis for susceptibility to noise-induced hearing loss in mice. Hear. Res. 155 (1–2), 82–90.

Dalton, D.S., Cruickshanks, K.J., Klein, B.E., Klein, R., Wiley, T.L., Nondahl, D.M., 2003. The impact of hearing loss on quality of life in older adults. Gerontologist 43, 661–668.

Dent, M.L., Screven, L.A., Kobrina, A., 2018. Hearing in rodents. In: Dent, M., Fay, R., Popper, A. (Eds.), Rodent Bioacoustics. Springer, Cham, Switzerland, pp. 71–105.

Diedenhofen, B., Musch, J., 2015. cocor: a comprehensive solution for the statistical comparison of correlations. PLoS One 10 (4), e0121945. doi:10.1371/journal.pone.0131499.

dos Santos Baraldi, G., de Almeida, L.C., Calais, L.L., de Carvalho Borges, A.C., Gielow, I., De Cunto, M.R., 2007. Study of the fundamental frequency in elderly women with hearing loss. Braz. J. Otorhinolaryngol. 73 (3), 378–383.

Egoscue, H.J., 1960. Laboratory and field studies of the northern grasshopper mouse. J. Mammal. 41 (1), 99–110.

Eliades, S.J., Wang, X., 2008. Neural substrates of vocalization feedback monitoring in primate auditory cortex. Nature 453 (7198), 1102–1106.

Emerich, K., Hoover, C., Sataloff, R., 1996. The effects of menopause on the singing voice. J. Singing 52, 39–42.

Erway, L.C., Shiau, Y.W., Davis, R.R., Krieg, E.F., 1996. Genetics of age-related hearing loss in mice. III. Susceptibility of inbred and F1 hybrid strains to noise-induced hearing loss. Hear. Res. 93 (1–2), 181–187.

Ferrand, C.T., 2002. Harmonics-to-noise ratio: an index of vocal aging. J. Voice 16 (4), 480–487.

Fröhlich, F., Ernst, A., Strübing, I., Basta, D., Gröschel, M., 2017. Apoptotic mechanisms after repeated noise trauma in the mouse medial geniculate body and primary auditory cortex. Exp. Brain Res. 235 (12), 3673–3682.

Gall, M., Brierley, L.E., Lucas, J.R., 2011. Species and sex effects on auditory processing in brown-headed cowbirds and red-winged blackbirds. Anim. Behav. 82, 973–982.

Gates, G.A., Cooper, J.C., 1991. Incidence of hearing decline in the elderly. Acta Otolaryngol. 111, 240–248.

Green, D.M., Scolman, T., Guthrie, O.W., Pasch, B., 2019. A broad filter between call frequency and peripheral auditory sensitivity in northern grasshopper mice (Onychomys leucogaster). J. Compar. Physiol. A 205 (4), 481–489.

Guthrie, O.W., 2017. Functional consequences of inducible genetic elements from the p53SOS response in a mammalian organ system. Exp. Cell Res. 359, 50–61.

Guthrie, O.W., Gearhart, C.A., Fulton, S., Fechter, L.D., 2011. Carboxy alkyl esters of Uncaria tomentosa augment recovery of sensorineural functions following noise injury. Brain Res. 1407, 97–106.

Guthrie, O.W., 2016. Preservation of neural sensitivity after noise-induced suppression of sensory function. J. Am. Acad. Audiol. 27, 49–61.

Guthrie, O.W., Xu, H., 2012. Noise exposure potentiates the subcellular distribution of nucleotide excision repair proteins within spiral ganglion neurons. Hear. Res. 294, 21–30.

Hafner, M.S., Hafner, D.J., 1979. Vocalizations of grasshopper mice (genus Onychomys). J. Mammal. 60 (1), 85–94.

Hammerschmidt, K., Reisinger, E., Westekemper, K., Ehrenreich, L., Strenzke, N., Fischer, J., 2012. Mice do not require auditory input for the normal development of their ultrasonic vocalizations. BMC Neurosci. 13 (1), 1–9.

Harel, B., Cannizzaro, M., Snyder, P.J., 2004. Variability in fundamental frequency during speech in prodromal and incipient Parkinson's disease: a longitudinal case study. Brain Cogn. 56 (1), 24–29.

Heffner, H.E., Heffner, R.S., 1985. Hearing in two cricetid rodents: wood rat (Neotoma floridana) and grasshopper mouse (Onychomys leucogaster). J. Comp. Psychol. 99 (3), 275–288.
Henry, K.R., 2004. Males lose hearing earlier in mouse models of late-onset age-related hearing loss; females lose hearing earlier in mouse models of early-onset hearing loss. Hear. Res. 190, 141–148.
Hederstierna, C., Hultcrantz, M., Collins, A., Rosenhall, U., 2010. The menopause triggers hearing decline in healthy women. Hear. Res. 259 (1–2), 31–35.
Higgins, M.B., McCleary, E.A., Carney, A.E., Schulte, L., 2003. Longitudinal changes in children's speech and voice physiology after cochlear implantation. Ear Hear. 24 (1), 48–70.
Higgins, M.B., McCleary, E.A., Ide-Helvie, D.L., Carney, A.E., 2005. Speech and voice physiology of children who are hard of hearing. Ear Hear. 26 (6), 546–558.
Hilliard, A.T., Miller, J.E., Fraley, E.R., Horvath, S., White, S.A., 2012. Molecular microcircuitry underlies functional specification in a basal ganglia circuit dedicated to vocal learning. Neuron 73 (3), 537–552.
Homans, N.C., Metselaar, R.M., Dingemanse, J.G., van der Schroeff, M.P., Brocaar, M.P., Wieringa, M.H., Goedegebure, A., 2017. Prevalence of age-related hearing loss, including sex differences, in older adults in a large cohort study. Laryngoscope 127 (3), 725–730. doi:10.1002/lary.26150.
Huang, Q., Tang, J., 2010. Age-related hearing loss or presbycusis. Eur. Arch. Otorhinolaryngol. 267 (8), 1179–1191.
Hubka, P., Konerding, W., Kral, A., 2015. Auditory feedback modulates development of kitten vocalizations. Cell Tissue Res. 361 (1), 279–294.
Hultcrantz, M., Simonoska, R., Stenberg, A.E., 2006. Estrogen and hearing: a summary of recent investigations. Acta Otolaryngol. 126 (1), 10–14.
Hulterantz, M., Li, H.S., 1993. Inner ear morphology in CBA/Ca and C57BL/6 J mice in relationship to noise, age and phenotype. Eur. Arch. Otorhinolaryngol. 250 (5), 257–264.
Jacobson, J.T., 1985. An overview of the auditory brainstem response. In: In: Jacobson J.T. (ed), The Auditory Brainstem Response. College-Hill Press, San Diego, pp. 3–12.
Johnson, A.M., Grant, L.M., Schallert, T., Ciucci, R.M., 2015. Changes in rat 50-kHz ultrasonic vocalizations during dopamine denervation and aging: relevance to neurodegeneration. Curr. Neuropharmacol. 13 (2), 211–219.
Kane, K.L., Longo-Guess, C.M., Gagnon, L.H., Ding, D., Salvi, R.J., Johnson, K.R., 2012. Genetic background effects on age-related hearing loss associated with Cdh23 variants in mice. Hear. Res. 283 (1–2), 80–88.
Kobrina, A., Dent, M.L., 2016. The effects of aging and sex on detection of ultrasonic vocalizations by adult CBA/CaJ mice (Mus musculus). Hear. Res. 341, 119–129. doi:10.1016/j.heares.2016.08.014.
Kobrina, A., Dent, M.L., 2019. The effects of age and sex on the detection of pure tones by adult CBA/CaJ mice (Mus musculus). J. Neurosci. Res. 98, 1731–1744. doi:10.1002/jnr.24496.
Kobrina, A., Schrode, K.M., Screven, L.A., Javaid, H., Weinberg, M.M., Brown, G., Lauer, A.M., 2020. Linking anatomical and physiological markers of auditory system degeneration with behavioral hearing assessments in a mouse (Mus musculus) model of age-related hearing loss. Neurobiol. Aging 96, 87–103. doi:10.1016/j.neurobiolaging.2020.08.012.
Kujawa, S.G., Liberman, M.C., 2009. Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J. Neurosci. 29 (45), 14077–14085. doi:10.1523/JNEUROSCI.2845-09.2009.
Lane, H., Matthies, M.L., Guenther, F.H., Denny, M., Perkell, J.S., Stockmann, E., Zandipour, M., 2007. Effects of short-and long-term changes in auditory feedback on vowel and sibilant contrasts. J. Speech Lang. Hear. Res. 50 (4), 913–927. doi:10.1044/1092-4388-2007/065.
Lane, H., Perkell, J.S., 2005. Control of voice-onset time in the absence of hearing: a review. J. Speech Lang. Hear. Res. 48, 1334–1343.
Lenth, R., 2020. EMMEANS: Estimated Marginal Means, Aka Least-Squares Means R package version 1.4.7 https://CRAN.R-project.org/package=emmeans .
Li-Korotky, H.S., 2012. Age-related hearing loss: quality of care for quality of life. Gerontologist 52 (2), 265–271.
Liberman, M.C., 2017. Noise-induced and age-related hearing loss: new perspectives and potential therapies. F1000Research 6, 927–938. doi:10.12688/f1000research.11310.1.
Liberman, M.C., Epstein, M.J., Cleveland, S.S., Wang, H., Maison, S.F., 2016. Toward a differential diagnosis of hidden hearing loss in humans. PloS One 11 (9), e0162726. doi:10.1371/journal.pone.0162726.
Lin, C.Y., Wu, J.L., Shih, T.S., Tsai, P.J., Sun, Y.M., Guo, Y.L., 2009. Glutathione S-transferase M1, T1, and P1 polymorphisms as susceptibility factors for noise-induced temporary threshold shift. Hear. Res. 257 (1–2), 8–15.
Lin, F.R., Ferrucci, L., Metter, E.J., An, Y., Zonderman, A.B., Resnick, S.M., 2011. Hearing loss and cognition in the Baltimore Longitudinal Study of Aging. Neuropsychology 25, 763–770. doi:10.1037/a0024238.
MATLAB (v. 2018a), 2019. Computer Sofware. The MathWorks Inc., Natick, Massachusetts.
Miller, J.R., Engstrom, M.D., 2012. Vocal stereotypy in the rodent genera Peromyscus and Onychomys (Neotominae): taxonomic signature and call design. Bioacoustics 21 (3), 193–213.
Moore, B.C., 2016. A review of the perceptual effects of hearing loss for frequencies above 3 kHz. Int. J. Audiol. 55 (12), 707–714.
Mora, R., Crippa, B., Cervoni, E., Santomauro, V., Guastini, L., 2012. Acoustic features of voice in patients with severe hearing loss. J. Otolaryngol. Head Neck Surg. 41 (1), 8–13.
Myint, A., White, C.H., Ohmen, J.D., Li, X., Wang, J., Lavinsky, J., Friedman, R.A., 2016. Large-scale phenotyping of noise-induced hearing loss in 100 strains of mice. Hear. Res. 332, 113–120.
Nicholas, J.G., Geers, A.E., 2006. Effects of early auditory experience on the spoken language of deaf children at 3 years of age. Ear Hear. 27 (3), 286–298.
Nordeen, K.W., Nordeen, E.J., 1992. Auditory feedback is necessary for the maintenance of stereotyped song in adult zebra finches. Behav. Neural Biol. 57 (1), 58–66.
Ohlemiller, K.K., 2006. Contributions of mouse models to understanding of age-and noise-related hearing loss. Brain Res. 1091 (1), 89–102.
Ohlemiller, K.K., Dahl, A.R., Gagnon, P.M., 2010. Divergent aging characteristics in CBA/J and CBA/CaJ mouse cochleae. J. Assoc. Res. Otolaryngol. 11, 605–623. doi:10.1007/s10162-010-0228-1.
Ohlemiller, K.K., Jones, S.M., Johnson, K.R., 2016. Application of mouse models to research in hearing and balance. J. Assoc. Res. Otolaryngol. 17, 493–523. doi:10.1007/s10162-016-0589-1.
Ohlemiller, K.K., 2018. Lessons from rodent models for genetic and age-related hearing loss. In: Dent, M., Fay, R., Popper, A. (Eds.), Rodent Bioacoustics. Springer, Cham, Switzerland, pp. 165–190.
Osmanski, M.S., Dooling, R.J., 2009. The effect of altered auditory feedback on control of vocal production in budgerigars (Melopsittacus undulatus). J. Acoust. Soc. Am. 126 (2), 911–919.
Pasch, B., Abbasi, M.Z., Wilson, M., Zhao, D., Searle, J.B., Webster, M.S., Rice, A.N., 2016. Cross-fostering alters advertisement vocalizations of grasshopper mice (Onychomys): evidence for the developmental stress hypothesis. Physiol. Behav. 157, 265–269.
Pasch, B., Tokuda, I.T., Riede, T., 2017. Grasshopper mice employ distinct vocal production mechanisms in different social contexts. Proc. Royal Soc. B: Biol. Sci. 284 (1859), 20171158.
Pearson, J.D., Morrell, C.H., Gordon-Salant, S., Brant, L.J., Metter, E.J., Klein, L.L., Fozard, J.L., 1995. Gender differences in a longitudinal study of age-associated hearing loss. J. Acoust. Soc. Am. 97, 1196–1205.
Perkell, J.S., Guenther, F.H., Lane, H., Matthies, M.L., Perrier, P., Vick, J., Zandipour, M., 2000. A theory of speech motor control and supporting data from speakers with normal hearing and with profound hearing loss. J. Phon. 28 (3), 233–272.
Peter, A.K., Crocini, C., Leinwand, L.A., 2017. Expanding our scientific horizons: utilization of unique model organisms in biological research. EMBO J. 36 (16), 2311–2314.
Peterson, J.R., Watts, C.R., Morris, J.A., Shelton, J.M., Cooper, B.G., 2013. Laryngeal aging and acoustic changes in male rat ultrasonic vocalizations. Dev. Psychobiol. 55 (8), 818–828.
R Core Team, 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria http://www.R-project.org/.
Radziwon, K.E., June, K.M., Stolzberg, D.J., Xu-Friedman, M.A., Salvi, R.J., Dent, M.L., 2009. Behaviorally measured audiograms and gap detection thresholds in CBA/CaJ mice. J. Compar. Physiol. A 195, 961–969.
Riede, T., 2013. Stereotypic laryngeal and respiratory motor patterns generate different call types in rat ultrasound vocalization. J. Exp. Zool. Part A: Ecol. Genet. Physiol. 319 (4), 213–224.
Riede, T., Borgard, H.L., Pasch, B., 2017. Laryngeal airway reconstruction indicates that rodent ultrasonic vocalizations are produced by an edge-tone mechanism. R. Soc. Open Sci. 4 (11), 170976.
Roy, N., Stemple, J., Merrill, R.M., Thomas, L., 2007. Epidemiology of voice disorders in the elderly: preliminary findings. Laryngoscope 117 (4), 628–633. doi:10.1097/MLG.0b013e3180306da1.
Ruffer, D.G., 1968. Agonistic behavior of the northern grasshopper mouse (Onychomys leucogaster breviauritus). J. Mammal. 49 (3), 481–487.
Shipley, C., Buchwald, J.S., Carterette, E.C., 1988. The role of auditory feedback in the vocalizations of cats. Exp. Brain Res. 69 (2), 431–438.
Smotherman, M.S., 2007. Sensory feedback control of mammalian vocalizations. Behav. Brain Res. 182 (2), 315–326.
Stapp, P., 1999. Size and habitat characteristics of home ranges of northern grasshopper mice (Onychomys leucogaster). Southwest. Nat. 44 (1), 101–105.
Tambs, K., Hoffman, H.J., Borchgrevink, H.M., Holmen, J., Samuelsen, S.O., 2003. Hearing loss induced by noise, ear infections, and head injuries: results from the Nord-Trøndelag hearing loss study. Int. J. Audiol. 42, 89–105.
Tschida, K., Mooney, R., 2012. The role of auditory feedback in vocal learning and maintenance. Curr. Opin. Neurobiol. 22 (2), 320–327.
Waldstein, R.S., 1990. Effects of postlingual deafness on speech production: implications for the role of auditory feedback. J. Acoust. Soc. Am. 88 (5), 2099–2114.
Zheng, Q.Y., Johnson, K.R., Erway, L.C., 1999. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear. Res. 130, 94–107. doi:10.1016/S0378-5955(99)00003-9.