Published in final edited form as:

Hear Res. 2020 January ; 385: 107845. doi:10.1016/j.heares.2019.107845.

Paired measurements of cochlear function and hair cell count in Dutch-belted rabbits with noise-induced hearing loss

Hariprakash Haragopal^a, Ryan Dorkoski^a, Holly M. Johnson^a, Mark A. Berryman^b, Soichi Tanda^a, Mitchell L. Day^{a,c,*}

^aDepartment of Biological Sciences, College of Arts and Sciences

^bDepartment of Biomedical Sciences, Heritage College of Osteopathic Medicine

^cQuantitative Biology Institute, Ohio University, Athens, OH 45701, USA

Abstract

The effects of noise-induced hearing loss have yet to be studied for the Dutch-belted strain of rabbits, which is the only strain that has been used in studies of the central auditory system. We measured auditory brainstem responses (ABRs), $2f_1-f_2$ distortion product otoacoustic emissions (DPOAEs), and counts of cochlear inner and outer hair cells (IHCs and OHCs, respectively) from confocal images of Myo7a-stained cochlear whole-mounts in unexposed and noise-overexposed, Dutch-belted, male and female rabbits in order to characterize cochlear function and structure under normal-hearing and hearing-loss conditions. Using an octave-band noise exposure centered at 750 Hz presented under isoflurane anesthesia, we found that a sound level of 133 dB SPL for 60 min was minimally sufficient to produce permanent ABR threshold shifts. Overexposure durations of 60 and 90 min caused median click-evoked ABR threshold shifts of 10 and 50 dB, respectively. Susceptibility to overexposure was highly variable across ears, but less variable across test frequencies within the same ear. ABR and DPOAE threshold shifts were smaller, on average, and more variable in male than female ears. Similarly, post-exposure survival of OHCs was higher, on average, and more variable in male than female ears. We paired post-exposure ABR and DPOAE threshold shift data with hair cell count data measured in the same ear at the same frequency and cochlear frequency location. ABR and DPOAE threshold shifts exhibited critical values of 46 and 18 dB, respectively, below which the majority of OHCs and IHCs survived and above which OHCs were wiped out while IHC survival was variable. Our data may be of use to researchers who wish to use Dutch-belted rabbits as a model for the effects of noise-induced hearing loss on the central auditory system.

*Corresponding author. 1 Ohio University, Athens, OH 45701, USA. daym1@ohio.edu (M.L. Day).

Conflict of interest

The authors declare no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

rabbit; noise-induced hearing loss; auditory brainstem response; distortion product otoacoustic emission; permanent threshold shift; hair cell

1. Introduction

The effects of noise overexposure on cochlear structure and function have been studied in many mammalian animal models, including but not limited to mice (Wang et al., 2002), rats (Chen et al., 2003), chinchillas (Hamernik et al., 1989), guinea pigs (Cody et al., 1983), macaques (Stebbins et al., 1979), and rabbits. Previous studies on rabbits were conducted on the New Zealand (Fahey et al., 2008; Franklin et al., 1991; Luebke et al., 2015) and chinchilla strains (Borg et al., 1983a; Borg et al., 1983b; Borg et al., 1989), but none on the Dutch-belted strain, which is the only strain of rabbit that has been and continues to be used in studies of the central auditory system (Blanks et al., 2007; Buechel et al., 2018; Carney et al., 2014; Chung et al., 2019; Day et al., 2016; Kim et al., 2015). Borg et al. (1995) previously reported rabbit strain differences in susceptibility to noise overexposure, with higher auditory brainstem response (ABR) threshold shifts resulting from the same overexposure in the New Zealand white strain as compared to the chinchilla strain. This indicates that overexposure parameters developed for one strain of rabbit do not necessarily produce the same degree of hearing loss in other strains. In the present study, we determined the appropriate noise overexposure parameters to produce permanent ABR threshold shifts in Dutch-belted rabbits. We first report data from unexposed, male and female rabbits, including analyses of ABR thresholds, peak amplitudes and latencies, and thresholds of $2f_1$ – f_2 distortion product otoacoustic emissions (DPOAEs). We then report the effect of noise overexposure on both ABR and DPOAE thresholds and survival of cochlear inner and outer hair cells (IHCs and OHCs, respectively). Finally, we paired same-ear threshold shift and hair cell count data to assess the relationship between the two measures in which hair cell survival could be predicted from threshold shifts.

Rabbits are valued by hearing scientists for a number of reasons. They are a mammalian model with an audible range that extends to relatively low frequencies (Heffner et al., 1980), comparable to humans (Poulsen et al., 2000). Importantly, they can discriminate interaural time differences at low frequencies (Ebert et al., 2008), which is the binaural cue that dominates sound source directional perception in humans (Wightman et al., 1992). Their behavior is docile, which makes them useful for awake, head-fixed single-unit recordings in the central auditory system where motion may disrupt data collection. In particular, the Dutch-belted strain is a smaller strain that is easy to handle and has been previously used in numerous studies of the central auditory system, as listed above. Our data may therefore be of use to researchers who wish to use Dutch-belted rabbits as a model for the effects of noise-induced hearing loss on the central auditory system.

2. Methods

2.1 Animals

A total of 27 purpose-bred, Dutch-belted rabbits were used in the study (7 males and 20 females; Covance). Of these, 24 were young adults between 4.5 and 8 months old, and three were adults aged 11, 19, and 29 months. All but three underwent noise overexposure.

Rabbits were housed singly or in pairs in the Animal Care Facility at Ohio University in a 12-hr light/12-hr dark cycle with lights on beginning at 06:00. All animal procedures were approved by the Ohio University Institutional Animal Care and Use Committee.

2.2 ABR and DPOAE Measurement

Rabbits were initially anesthetized with xylazine (6 mg/kg, s.c.) and ketamine (35 mg/kg, i.m.), then maintained with 1% isoflurane mixed with oxygen presented through a gas mask for the duration of the procedure. Glycopyrrolate (0.01 mg/kg, s.c.) was administered to prevent mucus blockage of the airways. Body temperature was monitored continuously and maintained with a microwaveable heating pad, or for the noise overexposure procedure (Section 2.3), a feedback-regulated, electric blanket. All procedures took place within a walk-in, double-walled, sound-attenuated chamber (ETS-Lindgren Acoustic Systems). ABRs and DPOAEs were measured from one ear in a single procedure, then in the other ear two days later. For a given ear, DPOAEs were always measured before ABRs (Mhatre et al., 2010). Postexposure measurements were made two weeks after the noise overexposure, by which time thresholds had likely settled to permanent levels (Borg et al., 1995).

Stimulus presentation and data acquisition were controlled by Eaton-Peabody Laboratories Cochlear Function Test Suite (CFTS) software running on a National Instruments (NI) chassis (PXIe-1082). Sound stimuli were created digitally, converted to analog signals by a 24-bit digital-to-analog converter at a sampling rate of 50 kHz (NI PXI-4461), and amplified (Tucker-Davis Technologies SA1) to drive an insert earphone (Etymotic ER-2). The sound tubes of two earphones (driven separately) and a probe tube microphone were embedded in a custom ear mold made from impression material (Reprosil), positioning the ends of all three tubes at the entrance of the ear canal. Sound was therefore presented in closed field. The probe tube microphone (Etymotic ER-7C) was used to both calibrate sound stimuli and measure DPOAEs. Measured acoustic signals were converted to digital signals at a sampling rate of 100 kHz (NI PXI-4461). ABRs were measured with platinum, subdermal needle electrodes (Grass Instruments) positioned at the vertex, mastoid (of the ear presented with sound), and back (common). Signals were amplified and filtered from 0.3 to 3 kHz (Grass P55), then converted to digital signals by a 16-bit analog-to-digital converter at a sampling rate of 25 kHz (NI PXIe-6341).

ABRs were measured in response to monaural, unipolar clicks (100 μ s) or tone pips (5-ms duration, 0.5-ms $\sin^2$ on/off ramps) at frequencies of 0.5 to 16 kHz in octave steps at 30 repetitions/s. The ABR at each sound level was averaged over 512 stimulus repetitions. For clicks and tone-pip frequencies at and above 2 kHz, stimulus polarity was alternated between successive repetitions to prevent contamination of the signal by the cochlear microphonic at high sound levels. Stimulus polarity was not alternated for 0.5- or 1-kHz

tones so that the phase-locked auditory nerve signal in Wave I was not eliminated. Stimuli were presented at sound levels from 10 to 75 dB SPL in 5-dB steps before noise overexposure and from 20 to 100 dB SPL after overexposure. ABR threshold was defined as the lowest sound level at which the average ABR first appeared, determined by visual inspection (Fig. 1A and B).

For off-line measurement of waveform peak amplitudes and latencies, average ABRs were additionally digitally filtered between 0.3 and 4 kHz with a 4-pole Butterworth filter. The first peak (Wave I) was defined as the maximum within 1.5–2.5 ms after stimulus onset. The following peak (Wave II) was defined as the next maximum after the trough following Wave I. Wave III was not prominent in most ABRs. Wave IV was defined as the maximum within 4.2–5 ms following stimulus onset. Wave V was defined as the next maximum after the trough following Wave IV. Peaks identified by this algorithm were inspected manually for accuracy. All data analyses were carried out in MATLAB (Mathworks).

DPOAEs were measured in response to two concurrent, 50-ms tones of frequencies f_1 and f_2 and sound levels L_1 and L_2 , respectively. Tones were presented from separate drivers to prevent the production of distortion by the acoustic system. Tones were presented with fixed frequency ratio of $f_2/f_1 = 1.2$ for f_2 frequencies of 2 to 16 kHz in octave steps. Each tone-frequency pair was presented at a fixed difference of levels $L_1 - L_2 = 10$ dB for L_2 levels of 10 to 75 dB SPL in 5-dB steps. L_2 was restricted to a maximum of 75 dB SPL to avoid distortion from nonphysiological sources (Kujawa et al., 2009). Acoustic responses were averaged over 1,024 stimulus repetitions for each combination of f_2 and L_2 , then the magnitude spectrum of the Fourier transform of the average acoustic response was computed. The amplitude of the $2f_1 - f_2$ DPOAE and surrounding noise floor were extracted from the magnitude spectrum (Fig. 1C). For each f_2 , DPOAE amplitude was plotted vs. L_2 (Fig. 1D); threshold was defined as the interpolated L_2 value at which the amplitude crossed the criterion value of 0 dB SPL. DPOAEs were not measured for an f_2 of 0.5 or 1 kHz because the noise floor was higher than the criterion amplitude; noise floor was below the criterion amplitude for all other frequencies.

Threshold shift following noise overexposure was computed as the difference of pre- and postexposure thresholds. For postexposure thresholds that exceeded the maximum sound level used (ABRs: 90–100 dB SPL depending on frequency and calibration; DPOAEs: 75 dB SPL), the minimum possible threshold shift was defined as the difference of pre-exposure threshold and maximum sound level presented. Data that are minimum possible threshold shifts are clearly labeled in figures.

2.3 Noise overexposure

Rabbits were anesthetized prior to noise overexposure, as described in Section 2.2, and monitored by video throughout the procedure. Noise waveforms were created in CFTS software and digitally filtered in an octave band geometrically centered at 750 Hz (530–1,061 Hz). Noise was converted to an analog signal at a sampling rate of 50 kHz (NI PXI-4461), amplified (Samson Servo 200), and produced by a pair of drivers (Selenium D250-X) coupled to exponential-shaped horns (Selenium HL14-25), one directed perpendicularly at each ear. The locations of the speakers relative to the rabbit were

reproduced for all rabbits. Noise waveforms presented from left and right speakers were independent, producing binaurally uncorrelated sound. This presentation method was used because some rabbits were utilized in a different study related to sound localization. Sound level was calibrated for each speaker by dangling the end of the probe tube microphone (using the -20 dB gain setting) at the approximate location of the center of the rabbit's head in the absence of the rabbit. Sound level when both speakers were presenting noise was 3 dB greater than when one speaker was presenting noise, which was expected from the sum of two independent noise waveforms. Noise overexposure level reported in the Results section is the sound level when both speakers were presenting sound. Several noise levels were tested between 122 and 135 dB SPL as detailed in Results.

2.4 Immunofluorescence

Following postexposure ABR and DPOAE measurements, rabbits were euthanized with pentobarbital (100 mg/kg, i.v.), then transcardially perfused with saline followed by 4% formaldehyde in phosphate buffered saline (PBS). Temporal bones containing the cochleae were immediately dissected. The oval and round windows were punctured, then the cochleae were gently perfused with 4% formaldehyde in PBS and post-fixed for 2 hrs at room temperature. Temporal bone fragments were decalcified in 0.25 M EDTA (pH 8.0) for several days, then cochleae were dissected in PBS. As previously described (Salles et al., 2014), the organ of Corti was microdissected from the rest of the cochlea and cut into pieces. Pieces were permeabilized for 30 min at room temperature in 0.5% Triton X-100 in PBS, then rinsed in PBS before blocking overnight in 4% BSA in PBS at 4°C. Pieces were then incubated for 15 hrs at 37°C in primary antibody against MYO7A (mouse anti-Myo7A antibody, DSHB, 1:100 dilution in 4% BSA/PBS), washed in PBS, then incubated for 1 hr at room temperature in secondary antibody (goat anti-mouse IgG conjugated with Alexa-488; Invitrogen Molecular Probes) and phalloidin (conjugated with Alexa-546; Invitrogen Molecular Probes). After washing, pieces were mounted in Prolong Gold (Invitrogen Molecular Probes). Cochlear pieces were then imaged with a confocal microscope (Nikon A1R) using a 10× objective lens. Cochlear hair cells were identified in maximum intensity projections of confocal z-stacks as cells positive for MYO7A, using phalloidin counter-staining as a marker for IHC and OHC stereocilia bundles (Hasson et al., 1995; Hasson et al., 1997).

2.5 Cochlear hair cell counts

The mapping between tone frequency and location along the cochlea with greatest sensitivity to that frequency was assumed to be the Greenwood function (Greenwood, 1990) modified for the upper frequency limit of rabbits (50 kHz) (Heffner et al., 1980): $f = 400(10^{2.1x} - 0.85)$, where f is the tone frequency in Hz and x is the fractional distance from apex. Confocal images of cochlear pieces were assembled from base to apex, then distance along the cochlea was measured along the cuticular plates of inner hair cells using Nikon NIS Elements software. Counts of both hair cells and empty “holes” where hair cells were missing were made within an area spanning 5% of the total cochlear distance centered at the cochlear places associated with each of the six tone frequencies used for ABR measurements. Counts were only made if at least half of the area about the cochlear frequency place was undisrupted by tissue folding or sectioning. Percent survival was

computed as the number of MYO7A-positive hair cells divided by the sum of the number of hair cells and holes. In tissue pieces where it was difficult to count the number of holes (e.g., Fig. 7C and D), the sum of the number of hair cells and holes was assumed to be equal to the average of that across rabbit ears at the same frequency place where hair cells and holes were countable.

2.6 Statistical analysis

To assess potential dependence of tone-evoked ABR and DPOAE thresholds of unexposed rabbits on tone-pip frequency and sex, ABR and DPOAE thresholds were each fit with a linear mixed-effects model with frequency, sex, and their interaction as fixed effects and individual ear as a random effect. Models were fit in MATLAB software via the function “fitlme” using restricted maximum likelihood estimation. Statistical significance of each fixed effect was determined using a *F*-test with a significance level of 0.05. If a fixed-effect term was significant, the model was re-fit using regular maximum likelihood estimation, then *post hoc* comparisons were made using *F*-tests, correcting for multiple comparisons using the Benjamini-Hochberg procedure.

Similarly, the amplitudes and latencies of the stereotypical peaks (or “waves”) in the average click-evoked ABR waveform of unexposed rabbits were each fit with a linear mixed-effects model with wave number, sex, and their interaction as fixed effects and individual ear as a random effect. ABR and DPOAE threshold shifts following noise overexposure were fit with a linear mixed-effects model with only frequency as a fixed effect and individual ear as a random effect. Models of threshold shifts were fit separately for male and female data because the variances of shifts were clearly different between the sexes.

3. Results

3.1 Functional assessments of cochleae in unexposed rabbits

We measured ABRs and DPOAEs from 27 rabbits of both sexes prior to noise overexposure. There was a small but significant sex difference of click-evoked ABR thresholds, with male thresholds on average 4 dB lower than female thresholds (*t*-test, $p = 0.006$, $N = 14$ male and 39 female ears) (Fig. 2A). Thresholds of tone-evoked ABRs were strongly dependent on tone-pip frequency, but not sex, with lowest thresholds between 4 and 16 kHz (*F*-test, frequency: $p = 4 \times 10^{-92}$, sex: $p = 0.11$, $N = 14$ male and 39 female ears). There was a small but significant interaction between frequency and sex due to a significant sex difference of thresholds only at 16 kHz (*F*-test, interaction: $p = 0.048$, male vs. female at 16 kHz: $p = 0.002$). Thresholds of DPOAEs were also strongly dependent on frequency with lowest thresholds between 4 and 16 kHz, but there was no significant effect of sex or frequency-sex interaction (*F*-test, frequency: $p = 1 \times 10^{-21}$, sex: $p = 0.80$, interaction: $p = 0.24$, $N = 14$ male and 16 female ears) (Fig. 2B). For both ABR and DPOAE thresholds, we fit a separate linear mixed-effects model with age as an additional fixed effect to test for potential variance in the data due to the inclusion of a minority of older rabbits. There was no significant effect of age on either ABR (*F*-test, $p = 0.19$) or DPOAE thresholds ($p = 0.07$).

In order to assess whether individual ears tended to have higher-than-average or lower-than-average thresholds across frequencies, we performed a within-ear, cross-frequency correlation analysis of ABR and DPOAE thresholds. Tables 1 and 2 show the correlation coefficients between thresholds measured in the same ear at two frequencies, for each possible pair of tone-pip frequencies. Most frequency pairs had significant, positive correlation, for both ABRs and DPOAEs. This means that ears with lower-than-average thresholds at one frequency tended to have lower-than-average thresholds at other frequencies, and vice versa for ears with higher-than-average thresholds. Further, ABR data had higher correlation coefficients for pairs closer in frequency; the same was not evident in DPOAE data. For example, the correlation coefficient between within-ear ABR thresholds at 0.5 and 1 kHz was 0.68 whereas that between thresholds at 0.5 and 16 kHz was 0.30. This means that ears with lower-than-average ABR thresholds at one frequency had a stronger tendency to have lower-than-average thresholds at nearby frequencies rather than distant frequencies.

In order to assess whether individual rabbits tended to have higher-than-average or lower-than-average thresholds between left and right ears, we performed an additional pairwise correlation analysis. We computed correlation coefficients from z-scored data in order to control for the frequency-dependence of ABR and DPOAE thresholds: for threshold values at each frequency, mean threshold was subtracted from the threshold values, then values were divided by their standard deviation. Figure 2C and D show scatterplots of z-scored left-ear vs. right-ear ABR and DPOAE thresholds, respectively, pooled across frequency. Both ABR and DPOAE z-scored thresholds were correlated between left and right ears. This means that there was a tendency for the threshold in one ear of a rabbit to be more similar to the threshold of its opposite ear than other rabbits' ears. In other words, both ABRs and DPOAEs were dependent between left and right ears even at the same frequency. We performed a similar correlation analysis between z-scored thresholds of ABRs and DPOAEs measured at the same ear in response to the same tone-pip frequency. Z-scored ABR and DPOAE thresholds were weakly correlated (Fig. 2E). This indicates there was only a weak tendency for a lower-than-average ABR threshold to have a lower-than-average DPOAE threshold at the same frequency, and vice versa for higher-than-average thresholds.

The ABR waveform contains a sequence of peaks, each of which originates from successive levels of the auditory pathway (Fig. 3A, Waves I–V). We measured the peak amplitudes and latencies of Waves I, II, IV and V from the average ABR in response to 70-dB SPL clicks (Wave III did not have a prominent peak). Wave I is of particular interest because its amplitude has been shown to be correlated with number of IHC ribbon synapses and may be used to infer synaptopathy following overexposures that cause temporary threshold shifts (Kujawa et al., 2009). Peak amplitudes varied substantially across ears and were best-fit by lognormal distributions. Log peak amplitude had a strong effect of wave number, but not sex, and significant interaction (*F*-test, wave number: $p = 7 \times 10^{-28}$, sex: $p = 0.87$, interaction: $p = 0.002$, $N = 14$ male and 39 female ears) (Fig. 3B). *Post hoc* tests revealed that the interaction of wave number and sex was complex. There were no significant sex differences within any wave—sex differences arose across wave number. However, log amplitude of Wave V was less than that of Waves I and II, regardless of sex. Log amplitude of Wave I was mildly correlated with that of Waves II, IV and V in the same ear, meaning there was a weak

tendency for an ear with a large-amplitude Wave I to also have large amplitudes for the other waves, and vice versa for small-amplitude waves (r [p] = 0.35 [0.01], 0.45 [7×10^{-4}], and 0.41 [0.002] for II/I, IV/I and V/I, respectively). Log amplitude was also correlated between left and right ears for all waves, indicating there was a tendency for peak amplitude in one ear of a rabbit to be more similar to that of its opposite ear than that of other rabbits' ears (r [p] = 0.68 [2×10^{-4}], 0.59 [0.002], 0.47 [0.016], and 0.43 [0.03] for Waves I, II, IV and V, respectively). There was no significant correlation between the log amplitude of Wave I at 70 dB SPL and click-evoked ABR threshold in the same ear (r = -0.13, p = 0.37).

Peak latency had a significant effect of sex, but no significant interaction between sex and wave number (the increase in latency with wave number is expected and trivial) (F -test, sex: p = 0.006, interaction: p = 0.11) (Fig. 3C). Female latencies lagged male latencies by 0.17 ms on average across wave number. The variability of peak latency was relatively small but increased with wave number, consistent with the accumulation of spike-timing variability through a sequence of synapses along the auditory pathway. Furthermore, the standard deviation of peak latencies was, on average, twice as large in females (0.18 ms) as in males (0.09 ms). Peak latencies were similar between left and right ears for all wave numbers, with correlation coefficients between 0.78 and 0.84 and regression line slopes between 0.995 and 1.05. For Waves II and V there was a tendency of higher amplitudes to have shorter latencies (Fig. 3D); the significance of this relationship is unclear. However, the same correlation was not seen in data from Waves I or IV.

Finally, we again fit peak amplitude and latency data with linear mixed-effects models that included age as a fixed effect. There was no significant effect of age on either log peak amplitude (F -test, p = 0.96) or peak latency (p = 0.95).

3.2 Functional assessments of cochleae in noise overexposed rabbits

In order to find noise overexposure parameters that would create permanent hearing loss but not profound deafness, we initially measured pilot pre- and postexposure ABR thresholds in female rabbits with different combinations of exposure level and duration. For a 15-min exposure duration, exposure levels of 122, 125, 129 and 133 dB SPL caused no threshold shift in response to clicks, while 134 and 135 dB SPL shifted thresholds above the limit of our acoustic system (~ 100 dB SPL). We next fixed exposure level at 133 dB SPL and varied exposure duration: median click-evoked threshold shifts were 0, 10 and 52.5 dB for durations of 30, 60 and 90 min, respectively.

In our first set of experiments, we exposed five female rabbits to noise at 133 dB SPL for 60 min. All of these rabbits were between 5 and 8 months old at the time of exposure. We measured pre- and postexposure thresholds for ABRs, but not DPOAEs. Postexposure click-evoked ABR thresholds (58 ± 10 dB SPL, mean $\pm$ SD) were highly variable compared to pre-exposure thresholds (44 ± 3 dB SPL), leading to a range of click-evoked threshold shifts from 0 to 30 dB with median 10 dB (Fig. 4A and B). Median tone-evoked threshold shifts were similar across frequency at around 18 dB. However, variability of postexposure tone-evoked thresholds was greater between 4 and 16 kHz, which led to large variability in threshold shifts in this frequency range. Data points in Figure 4B connected by same-colored lines indicate thresholds measured from the same ear. By following lines of the same color

in the upper frequency range (2–16 kHz), one can see that only two ears had threshold shifts that were clearly consistent across frequency. Nonetheless, variability of individual-ear susceptibility was greater than variability of threshold shifts across frequency within the same ear; 62% of the total variance in threshold shifts between 2 and 16 kHz could be explained by differences in mean threshold shift across individual ears. Altogether, while thresholds shifts were similar, on average, across frequencies, they were less variable across ears for frequencies associated with apical cochlear locations near our exposure band (0.53–1.1 kHz), and more variable for frequencies associated with cochlear locations basal to the exposure band.

In our second set of experiments, we exposed five female and five male rabbits to noise at 133 dB SPL for 90 min. All of these rabbits were between 5 and 8 months old at the time of exposure, except for two females aged 12 and 29 months. We measured pre- and postexposure thresholds for both ABRs and DPOAEs. In practice, thresholds of postexposure ABRs could not be identified for 0.5- and 1-kHz tone pips because the cochlear microphonic overwhelmed the signal at sound levels lower than that at which the ABR appeared (see Section 2.2). Since ABR thresholds at 0.5 and 1 kHz in unexposed rabbits are around 50 dB SPL and the cochlear microphonic appears at about 70 dB SPL at these frequencies, the threshold shifts at 0.5 and 1 kHz were therefore greater than 20 dB. Figure 5 shows ABR and DPOAE threshold shifts in response to clicks and tone pips at the upper frequencies (2–16 kHz), separated by sex. Median click-evoked ABR threshold shift in females was 55 dB—much greater than that following a 60-min overexposure (10 dB; Fig. 5B and 4B; females only). For some stimuli, postexposure ABR or DPOAE thresholds were above the limit of our acoustic system; such cases are labeled by solid squares in Figure 5 indicating minimum possible threshold shifts.

The most striking characteristic of our data was the difference in threshold shifts between males and females following the same 90-min overexposure, for both ABRs and DPOAEs. Whereas median click-evoked ABR threshold shifts were similar between males and females (50 and 55 dB, respectively), the range of click-evoked threshold shifts for males spanned substantially lower than that for females: 5–65 dB for males and 45–60 dB for females. The same expansion to lower threshold shifts in males was also observed for tone-evoked ABR threshold shifts and DPOAE threshold shifts. Lines of the same color in Figure 5 link data points from the same ear; those male ears that had smaller threshold shifts (either ABR or DPOAE) at one tone frequency had smaller threshold shifts at the other frequencies. Furthermore, the three male ears with smallest click-evoked threshold shifts came from three different rabbits; the other ears of these three rabbits had higher threshold shifts. Therefore, some male ears were less susceptible to noise overexposure at all frequencies (2–16 kHz), and an individual male rabbit may have one highly susceptible ear and one less susceptible ear. The large variability of susceptibility in males was not due to differences in age at the time of overexposure because male rabbits were homogeneous in age whereas female rabbits included two individuals exposed at older ages.

There was a weak effect of frequency on tone-evoked ABR threshold shifts of males (F -test, $p = 0.004$) due to a statistically significant difference in shifts between 2 and 4 kHz (F -test, $p = 0.0007$), but no effect of frequency on that of females (F -test, $p = 0.06$). Although

variability of tone-evoked ABR threshold shifts in females was less than that in males, it was still relatively large ($SD = 12$ dB over 2–16 kHz) and similar to that for females following a 60-min overexposure ($SD = 13$ dB over 2–16 kHz). The variability of tone-evoked ABR threshold shifts was dominated by variability across ears rather than variability across frequency within the same ear; 83% of the total variance in threshold shifts could be explained by differences in mean threshold shifts across ears. For DPOAEs, threshold shifts systematically increased with frequency for females (F -test, $p = 6 \times 10^{-7}$), but not males.

In Figure 6A and B, we plot threshold shifts in response to the same tone-pip frequency for left vs. right ears for ABRs and DPOAEs, respectively. Left- and right-ear threshold shifts were correlated for female rabbits for both ABRs and DPOAEs (ABR: $r = 0.69$, $p = 8 \times 10^{-4}$, $N = 20$; DPOAE: $r = 0.79$, $p = 3 \times 10^{-4}$, $N = 16$). In Figure 5D, we showed that DPOAE threshold shifts in female ears systematically increased with tone frequency. This raises the possibility that the correlation of left and right female DPOAE threshold shifts may be simply due to dependence on frequency. Sample sizes at each frequency were too small to accurately estimate z-scores, so we could not rule out this possibility. Male rabbits, on the other hand, often had highly asymmetric threshold shifts between ears, for both ABRs and DPOAEs. Three of the five male rabbits had an asymmetry of ABR threshold shifts of 15 dB or more for at least one tone frequency.

In Figure 6C, we plot ABR threshold shifts vs. DPOAE threshold shifts measured in the same ear in response to the same tone-pip frequency. ABR and DPOAE threshold shifts were correlated ($r = 0.75$, $p = 7 \times 10^{-14}$, $N = 72$). Most data points fell below the unity line, indicating ABR thresholds shifted by a greater amount than DPOAE thresholds.

3.3 Cochlear hair cell counts in unexposed and noise overexposed rabbits

In order to assess how noise overexposure affected the structural integrity of the cochlea, we counted the number of cochlear hair cells in nine rabbits, including three males and three females following overexposure, and two females and one male unexposed to noise (controls). Hair cell counts were restricted to the tonotopic regions of the cochlea associated with threshold activity of each of the six tone frequencies used in ABR measurements (0.5 to 16 kHz in octave steps). Figure 7A shows a cochlear micrograph from an unexposed rabbit; one row of inner hair cells and three rows of outer hair cells are clearly visible. The rest of the panels in Figure 7 show increasing levels of damage following noise overexposure from left to right: a partial loss of OHCs with completely intact IHCs (B), complete wipe-out of OHCs with mostly intact IHCs (C), and complete wipe-out of both IHCs and OHCs (D). Figure 8 shows the percent survival of hair cells vs. cochlear frequency place for the six unexposed, control ears. At least 92% of IHCs and 78% of OHCs survived across all frequency locations. The two ears with lowest OHC counts at 1 and 2 kHz were from the same female rabbit.

Figure 9 shows the percent survival of IHCs and OHCs following a noise overexposure of 133 dB SPL for 90 min, separated by sex. OHC and IHC survival in exposed ears was clearly lower than in unexposed ears, although not consistently across ears. OHC survival was dramatically affected by overexposure: only one ear (male) was unaffected by the exposure. OHC survival in female ears was mostly below 30% and largely consistent across

cochlear frequency place (Fig. 9A). On the other hand, OHC survival in male ears across cochlear frequency locations was complex: two ears had less than 20% survival across frequencies (OHC wipe-out), two ears had survival mostly above 80% across frequencies, one ear had OHC wipe-out at and above 2 kHz, and the last ear had OHC wipe-out at and below 8 kHz (Fig. 9B). There were no significant differences in OHC survival between the three OHC rows when compared at the same cochlear frequency places (Wilcoxon signed-rank test, $p = 0.95, 0.85$ and 0.41 for OHC row 1 vs. 2, 1 vs. 3, and 2 vs. 3, respectively). The overall effect of overexposure on OHCs was similar to that of ABR and DPOAE threshold shifts (Fig. 5) in that female ears were strongly and consistently susceptible while damage to male ears was highly variable. The effect of overexposure on IHC survival was less than that for OHCs and did not display the same obvious sex differences. Unlike OHCs, IHC survival was variable across ears and across cochlear frequency locations within an ear for both males and females (Fig. 9C and D).

In Figure 10, we plot a 2D histogram of percent IHC and OHC survival following noise overexposure occurring at the same cochlear frequency place within the same ear. Data are contained within the upper and left sides of the plot with a complete absence of points in the lower right corner, meaning IHC wipe-out never co-occurred with majority OHC survival at the same place in the cochlea. Data points in the upper right corner represent cochlear locations where most IHCs and OHCs survived following overexposure. If overexposure affected IHCs and OHCs to the same degree, we would expect the remaining points to fall along the diagonal line. Instead, data indicate an order to the severity of hair cell loss: cochlear locations either had 1) varying amounts of OHC loss with IHCs remaining largely intact (upper-right to upper-left corner), or 2) OHC wipe-out with varying amounts of IHC loss (upper-left to lower-left corner). These data indicate that rabbit OHCs are more susceptible to noise damage than IHCs.

3.4 Comparison of cochlear functional measures to hair cell counts

The six rabbits whose cochlear hair cell counts were described above (133 dB SPL, 90-min exposure) were a subset of the rabbits reported in Section 3.2 on postexposure ABR and DPOAE measurements. Therefore, we had paired measurements with which to observe potential relationships between ABR or DPOAE threshold shifts and hair cell counts. Figure 11 shows tone-evoked ABR or DPOAE threshold shifts vs. percent OHC or IHC survival. ABR or DPOAE data in response to a particular tone frequency was paired with hair cell count data from the same ear and at the cochlear frequency place matching that of the tone. For both ABRs and DPOAEs, OHC survival data were characterized by a critical threshold shift, below or above which most OHCs alternatively survived or died, respectively. We computed the critical threshold shifts that classified OHC data points into either greater or lesser than 50% survival with least error: threshold shifts of 46 dB for ABR and 18 dB for DPOAE (dashed lines in Fig. 11). This pattern was evident for data from male ears (red symbols) but not female ears (blue symbols) because male ears had threshold shift values that straddled the critical threshold shifts. However, one data point each for ABRs and DPOAEs from female ears was separated from the rest of the data and occurred near the critical threshold shifts. This suggests that the critical threshold shifts predicting OHC survival may not depend on sex. Included in the figures are data points indicating minimum

possible threshold shifts (crosses); nearly all these data were associated with cochlear locations with OHC survival of 20% or less. Although there were fewer data points below the critical threshold shifts, there was a trend of OHC survival decreasing gradually with increasing ABR threshold shift (Fig. 11A). On the other hand, DPOAE threshold shift was not useful in predicting gradations of OHC survival below the critical threshold shift (Fig. 11B).

IHC survival data were also characterized by critical threshold shifts for both ABRs and DPOAEs, below which there was greater than 90% survival, and above which there was a large range of percent survival spanning 5% to 100%. The transition at the critical threshold shifts was less pronounced than that for OHCs but corresponded well with the critical threshold shift values computed for OHCs (Fig. 11C and D). Altogether, ABR or DPOAE threshold shifts below their critical values strongly predicted corresponding cochlear locations with majority survival of OHCs and IHCs, whereas threshold shifts above their critical values strongly predicted corresponding cochlear locations with majority death of OHCs and variable survival of IHCs.

4. Discussion

4.1 Data from unexposed rabbits: comparison to other strains and species

Click-evoked ABR thresholds in our Dutch-belted rabbits (42 dB SPL) were higher than those reported for New Zealand white rabbits (35 dB p.e. SPL) (Stieve et al., 2006), rats (30 dB SPL) (Popelar et al., 2003), and mice (34 dB SPL) (Zhou et al., 2006), but similar to gerbils (40–45 dB SPL) (Burkard et al., 1989) and chinchillas (43 dB SPL) (Janning et al., 1998). The dependence of tone-evoked ABR thresholds on frequency was similar to that of the chinchilla strain of rabbits (Borg et al., 1983b) in that higher frequencies had lower thresholds (Fig. 2A). However, ABR thresholds at 0.5 kHz were higher in the chinchilla strain than in our Dutch-belted rabbits, which suggests that the Dutch-belted strain may have more sensitive low-frequency hearing. Behavioral thresholds of the chinchilla strain of rabbits mimicked a shifted version of their ABR thresholds (Borg et al., 1983b), but behavioral thresholds of the New Zealand strain of rabbits had most sensitive thresholds down to 1 kHz (Heffner et al., 1980), which suggests that their low-frequency hearing may be more sensitive than that of the Dutch-belted strain (Fig. 2A).

We found that thresholds were correlated between tone frequencies within the same ear, which implies that some ears are, overall, more or less sensitive to sound than other ears. Further, thresholds were mildly correlated between left and right ears. Together, these results suggest that some individual rabbits, and not simply individual ears, are more or less sensitive to sound than other rabbits.

We repeatedly observed Waves I, II, IV and V in click-evoked ABR waveforms of our Dutch-belted rabbits, with Wave III most often obscured, whereas Stieve et al. (2006) most often observed Waves I–IV in New Zealand rabbits, with Wave V often unobservable. In both strains of rabbit, peak amplitude decreased with wave number—a pattern similar to mice (Zhou et al., 2006), cats (Melcher et al., 1996), guinea pigs (Wada et al., 1983), and chinchillas (Henry et al., 2011), but in contrast to humans, in which the amplitude of Wave

V is larger than that of Wave I (Verhulst et al., 2016). In contrast to our finding that the variability of peak latency increased with wave number in Dutch-belted rabbits, Stieve et al. (2006) found constant, very low variability of peak latency across wave number in New Zealand rabbits, potentially due to their use of a higher-level click to evoke the ABR.

4.2 Threshold shifts following noise overexposure: comparison to other strains and species

Following acoustic overexposure, greatest permanent threshold shifts tend to occur at frequencies within and above the spectrum of the insulting sound, with corresponding damage to the cochlea at frequency places within and basal to the spectrum (Borg et al., 1995; Harding et al., 2007). Our aim was to use a noise that would cause widespread damage across the cochlea, including both basal and apical regions. Therefore, we centered our octave-band noise at 750 Hz (0.53–1.1 kHz), which corresponds to a cochlear frequency place near the apex in rabbits. We found that a sound level of 133 dB SPL for a duration of at least 60 min was sufficient to produce permanent threshold shifts. ABR threshold shifts and IHC and OHC survival were all similar across frequency, on average, indicating widespread damage of the cochlea was achieved; however, variability across ears was substantial. Our overexposure protocol may therefore be of use to researchers who wish to measure central auditory function in Dutch-belted rabbits following damage to both basal and apical regions of the cochlea.

In previous extensive studies by Borg et al. (1995) on the chinchilla strain of rabbits, a noise of only 125 dB SPL and 15 min duration was sufficient to produce permanent ABR threshold shifts. Their octave-band noise exposure was centered at 2.8 kHz (2–4 kHz), which partially overlaps with the range of most sensitive hearing in rabbits, while our exposure was centered in a less sensitive range. The difference in normal-hearing ABR thresholds between the two center frequencies of exposure is approximately 15 dB (Fig. 2A). Borg et al. (1995) also found that anesthesia increased threshold shifts for the same exposure and decreased the “critical” sound level sufficient to produce threshold shifts. Their critical sound level of 125 dB SPL was measured in response to awake overexposures and is likely higher than that if measured in response to anesthetized exposures, as in our study. Together, differences in exposure spectrum and arousal state may account for the difference in critical sound level sufficient to produce permanent threshold shifts. On the other hand, Borg et al. (1995) also found that chinchilla and albino strains of rabbits differed in ABR threshold shifts by as much as 10 dB following the same overexposure. The higher critical sound level found in our Dutch-belted rabbits may therefore also be due in part to differences in strain. Of particular note is our finding that, at a duration of 15 min, permanent ABR threshold shifts went from zero at 133 dB SPL to extremely high at 134 dB SPL, whereas Borg et al. observed in the chinchilla strain for the same exposure duration a graded increase in threshold shifts with incremental increases in sound level over a 15-dB range. Again, a potential confound between the two studies may be the arousal state of the rabbits during overexposure. Anesthesia is known to suppress the middle-ear muscle reflex (Borg et al., 1975; Borg et al., 1983c).

Noise overexposure levels lower than that used in our study were sufficient to produce permanent threshold shifts in other species, including mice (110 dB SPL) (Erway et al., 1996), chinchillas (115 dB SPL) (Henry et al., 2011; Strominger et al., 1995), cats (111 dB SPL) (Ngan et al., 2001), and guinea pigs (114 dB SPL) (McFadden et al., 2005). However, in all of these studies the spectrum of the exposure band was positioned within the most sensitive hearing range, whereas in our study the exposure band was below the most sensitive range, as described above. In contrast to our study, macaques required a greater noise overexposure of 140 dB SPL to produce permanent threshold shifts using a 50-Hz-wide band centered at 2 kHz (Valero et al., 2017).

ABR threshold shifts following our noise overexposure were highly variable across ears—dramatically so for males but also substantially for females. This variability was quantitatively similar to that shown previously for the chinchilla strain of rabbits following noise overexposure (Borg et al., 1995). Similar variability in susceptibility to noise overexposure has also been reported in guinea pigs (Cody et al., 1983). Differences in individual susceptibility to overexposure may be partially due to genetic factors involved in regulating reactive oxygen species or maintaining the structural integrity of the organ of Corti (Konings et al., 2009). Such genetic factors would explain variability across individuals but not variability between ears of the same individual.

4.3 OHC susceptibility and critical threshold shifts

We found that OHC survival was more susceptible to noise overexposure than IHC survival, consistent with data from many species, including humans (Borg et al., 1995; Chen et al., 2003; Harding et al., 2004; Rask-Andersen et al., 2000; Stebbins et al., 1979). The dominant factor that makes OHCs more susceptible to overexposure than IHCs is unknown, but may be due to a difference in Ca^{2+} homeostasis (Fettiplace et al., 2018). On the other hand, widespread IHC loss with only moderate OHC loss has been reported in the chinchilla strain of rabbits in response to 15 min of noise overexposure at 134 dB SPL (Borg et al., 1995).

It is well-known that complete OHC loss is linked with highly elevated thresholds (Borg et al., 1983b; Dallos et al., 1978). Our data indicated critical ABR and DPOAE threshold shifts above which OHCs transitioned from majority survival to near complete wipe-out (Fig. 11A and B). The existence of a sharp, critical threshold shift for OHC loss is not common in the literature. In chinchillas and rats, no sharp, critical threshold shift was observed for ABRs or DPOAEs, but for some frequencies the relationship between OHC loss and threshold shift was linear (Chen et al., 2003; Hamernik et al., 2000). In our data, threshold shifts below the critical threshold shift were not correlated with OHC survival. Shifts in ABR or DPOAE thresholds without loss of OHCs were likely due to stereocilia damage. Stereocilia of IHCs in the chinchilla strain of rabbit have been shown to become damaged without loss of OHCs up to an ABR threshold shift of 40 dB (Borg et al., 1995). In our histological preparations of overexposed cochleae, we observed some hair cells with faint Myo-7A staining and counted them as surviving hair cells. It is unclear whether or not such faint staining was due to dysfunction of the hair cells.

Above the critical threshold shift, IHC survival was variable and could not be predicted from threshold shifts (Fig. 11C and D). A previous study in chinchillas reported that behavioral

thresholds were insensitive to specific loss of IHCs exceeding 80% (Lobarinas et al., 2013). This suggests that thresholds measured in our data were unrelated to IHC survival.

4.4 Sex difference in susceptibility to noise overexposure

We found that some male ears were less susceptible to noise overexposure than female ears (Fig. 5). Previous studies have also reported sex differences in susceptibility to overexposure, but not always in the same direction. In the chinchilla strain of rabbits, male ears were more susceptible to overexposure than female ears (Borg et al., 1995), while in chinchillas, sex differences in susceptibility were flipped between the low- and high-frequency hearing ranges (McFadden et al., 1999). Sex differences in susceptibility have also been found in some strains of mice (Milon et al., 2018), but not others (Erway et al., 1996; Kujawa et al., 2006). In our data, it is unlikely that the sex difference in susceptibility was due to differences that would affect both ears, like body mass or head size, because threshold shifts were asymmetric between the left and right ears of three of the five male rabbits (Fig. 6A and B). Loudspeakers and rabbit head placement were in the same position during noise overexposure procedures for all rabbits, therefore the possibility of off-center head placement between the speakers was unlikely; furthermore, repeated off-center placement would not be expected to affect males and not females. We did not inspect ear canals for obstruction before noise overexposures; however, such obstruction would have affected measurements of pre-exposure thresholds.

4.5 Comparison of ABR and DPOAE thresholds

ABR and DPOAE thresholds are thought to be predominately influenced by OHC function even though the two signals arise from different sources of generation (Borg et al., 1983b; Hamernik et al., 2000; Ohlms et al., 1991; Probst et al., 1991). Consistent with this, unexposed ears in our data set had quantitatively similar frequency-dependent ABR and DPOAE thresholds, on average (Fig. 2A and B). On the other hand, we found only weak correlation between ABR and DPOAE thresholds matched for ear and frequency (Fig. 2E). Similar weak correlation between ABR and DPOAE thresholds has been reported in macaques (Lasky et al., 1999). One possibility for such weak correlation may be that potentially strongly correlated measures were swamped by measurement error. We did not implement a test/retest protocol to estimate measurement error; however, a previous estimate of error in measurement of ABR thresholds in the chinchilla strain of rabbits had a SD of 5 dB (Borg et al., 1995), which is equal to the across-ear SD of ABR thresholds in our study. Another possible explanation for weak correlation between ABR and DPOAE thresholds may be due to the sources of generation of DPOAEs; one source arises from the same location along the basilar membrane as that of the ABR, but the other source arises from a more apical location (reviewed in Shaffer et al., 2003; Shera, 2004).

We found that post-exposure ABR threshold shifts were greater and more variable than DPOAE threshold shifts (Fig. 6C). This was not due to a ceiling effect of the range of sound levels tested because there were more data that were minimum possible threshold shifts for ABRs than for DPOAEs (Fig. 5). We also found that ABR threshold shifts were correlated with DPOAE threshold shifts. Similar correlation between the two post-exposure shifts has been reported in gerbils (Mills, 2003).

Our results indicate that ABR and DPOAE threshold shifts were equivalently useful in predicting majority OHC survival or OHC wipe-out (Fig. 11A and B). Similarly, they could both be used to predict majority survival of IHCs vs. potential loss of IHCs (Fig. 11C and D). Knowledge of both threshold shifts did not increase predictability of survival for either OHCs or IHCs—with respect to predicting OHC or IHC survival, the two measures were redundant.

5. Conclusions

- ABR and DPOAE thresholds were similar, on average, in unexposed ears and had highest sensitivity between 4 and 16 kHz. Thresholds from the same ear were correlated between frequencies.
- ABRs in unexposed ears had equivalent amplitudes of Waves I and II and a decreased amplitude of Wave V.
- An octave-band noise overexposure centered at 750 Hz of 133 dB SPL for 60 min was minimally sufficient to produce permanent threshold shifts. The relatively high sufficient level of exposure may be due to the exposure band being positioned in a less-sensitive portion of the hearing range.
- Noise overexposure produced ABR threshold shifts that were similar, on average, across frequency. Susceptibility was highly variable across ears. Male ears were less susceptible and more variable in ABR and DPOAE threshold shifts than female ears.
- OHC survival was more susceptible to overexposure than IHC survival. OHC survival was low in female ears and highly variable in male ears, similar to the pattern observed for ABR and DPOAE threshold shifts. IHC survival was mostly high but varied across ears.
- ABR and DPOAE threshold shifts exhibited respective critical values of 46 and 18 dB, above which OHCs at the corresponding cochlear frequency location sharply transitioned from majority survival to wipe-out. Above the same critical value, IHCs transitioned from majority survival to varying amounts of survival.

Acknowledgements

This research was supported by the National Institute on Deafness and Other Communication Disorders of the National Institutes of Health (NIH) under Award Numbers R15DC017616 (M.D.), R03DC013388 (M.D.), R15DC017032 (M.B., S.T., M.D.), and an internal award from Ohio University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. We thank Ken Hancock for software support, and acknowledge use of the Ohio University Heritage College Microscopy Core.

References

- Blanks DA, Roberts JM, Buss E, Hall JW, Fitzpatrick DC 2007 Neural and behavioral sensitivity to interaural time differences using amplitude modulated tones with mismatched carrier frequencies. *J Assoc Res Otolaryngol* 8, 393–408. [PubMed: 17657543]
- Borg E, Moller AR 1975 Effect of central depressants on the acoustic middle ear reflex in rabbit. A method for quantitative measurements of drug effect on the CNS. *Acta Physiol Scand* 94, 327–38. [PubMed: 1180078]

- Borg E, Engström B 1983a Damage to sensory hairs of inner hair cells after exposure to noise in rabbits without outer hair cells. *Hearing research* 11, 1–6. [PubMed: 6885646]
- Borg E, Engstrom B 1983b Hearing thresholds in the rabbit. A behavioral and electrophysiological study. *Acta Oto-Laryngol* 95, 19–26.
- Borg E, Engström B 1989 Noise level, inner hair cell damage, audiometric features, and equal-energy hypothesis. *The Journal of the Acoustical Society of America* 86, 1776–1782. [PubMed: 2808926]
- Borg E, Nilsson R, Engstrom B 1983c Effect of the Acoustic Reflex on Inner-Ear Damage Induced by Industrial Noise. *Acta Oto-Laryngol* 96, 361–369.
- Borg E, Canlon B, Engström B 1995 Noise-induced hearing loss. Literature review and experiments in rabbits. Morphological and electrophysiological features, exposure parameters and temporal factors, variability and interactions. *Scandinavian audiology. Supplementum* 40, 1.
- Buechel BD, Hancock KE, Chung Y, Delgutte B 2018 Improved Neural Coding of ITD with Bilateral Cochlear Implants by Introducing Short Inter-pulse Intervals. *Jaro-J Assoc Res Oto* 19, 681–702.
- Burkard R, Voigt HF 1989 Stimulus dependencies of the gerbil brain-stem auditory-evoked response (BAER). I: Effects of click level, rate, and polarity. *The Journal of the Acoustical Society of America* 85, 2514–2525. [PubMed: 2745876]
- Carney LH, Zilany MSA, Huang NJ, Abrams KS, Idrobo F 2014 Suboptimal Use of Neural Information in a Mammalian Auditory System. *Journal of Neuroscience* 34, 1306–1313. [PubMed: 24453321]
- Chen G-D, Fechter LD 2003 The relationship between noise-induced hearing loss and hair cell loss in rats. *Hearing research* 177, 81–90. [PubMed: 12618320]
- Chung YJ, Buechel BD, Sunwoo W, Wagner JD, Delgutte B 2019 Neural ITD Sensitivity and Temporal Coding with Cochlear Implants in an Animal Model of Early-Onset Deafness. *Jaro-J Assoc Res Oto* 20, 37–56.
- Cody A, Robertson D 1983 Variability of noise-induced damage in the guinea pig cochlea: electrophysiological and morphological correlates after strictly controlled exposures. *Hearing research* 9, 55–70. [PubMed: 6826468]
- Dallos P, Harris D 1978 Properties of auditory nerve responses in absence of outer hair cells. *J Neurophysiol* 41, 365–83. [PubMed: 650272]
- Day ML, Delgutte B 2016 Neural population encoding and decoding of sound source location across sound level in the rabbit inferior colliculus. *J Neurophysiol* 115, 193–207. [PubMed: 26490292]
- Ebert CS Jr., Blanks DA, Patel MR, Coffey CS, Marshall AF, Fitzpatrick DC 2008 Behavioral sensitivity to interaural time differences in the rabbit. *Hear Res* 235, 134–42. [PubMed: 18093767]
- Erway LC, Shiau YW, Davis RR, Krieg EF 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, 181–7. [PubMed: 8735078]
- Fahey PF, Stagner BB, Martin GK 2008 Source of level dependent minima in rabbit distortion product otoacoustic emissions. *Journal of the Acoustical Society of America* 124, 3694–3707. [PubMed: 19206797]
- Fettiplace R, Nam JH 2018 Tonotopy in calcium homeostasis and vulnerability of cochlear hair cells. *Hear Res.*
- Franklin DJ, Lonsbury-Martin BL, Stagner BB, Martin GK 1991 Altered susceptibility of 2f1–f2 acoustic-distortion products to the effects of repeated noise exposure in rabbits. *Hearing research* 53, 185–208. [PubMed: 1880074]
- Greenwood DD 1990 A cochlear frequency-position function for several species--29 years later. *J Acoust Soc Am* 87, 2592–605. [PubMed: 2373794]
- Hamernik RP, Qiu W 2000 Correlations among evoked potential thresholds, distortion product otoacoustic emissions and hair cell loss following various noise exposures in the chinchilla. *Hearing Research* 150, 245–257. [PubMed: 11077207]
- Hamernik RP, Patterson JH, Turrentine GA, Ahroon WA 1989 The quantitative relation between sensory cell loss and hearing thresholds. *Hearing research* 38, 199–211. [PubMed: 2708163]
- Harding GW, Bohne BA 2004 Noise-induced hair-cell loss and total exposure energy: analysis of a large data set. *J Acoust Soc Am* 115, 2207–20. [PubMed: 15139632]

- Harding GW, Bohne BA 2007 Distribution of focal lesions in the chinchilla organ of Corti following exposure to a 4-kHz or a 0.5-kHz octave band of noise. *Hear Res* 225, 50–9. [PubMed: 17291699]
- Hasson T, Heintzelman MB, Santos-Sacchi J, Corey DP, Mooseker MS 1995 Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B. *Proc Natl Acad Sci U S A* 92, 9815–9. [PubMed: 7568224]
- Hasson T, Gillespie PG, Garcia JA, MacDonald RB, Zhao Y, Yee AG, Mooseker MS, Corey DP 1997 Unconventional myosins in inner-ear sensory epithelia. *J Cell Biol* 137, 1287–307. [PubMed: 9182663]
- Heffner H, Masterton B 1980 Hearing in Glires - Domestic Rabbit, Cotton Rat, Feral House Mouse, and Kangaroo Rat. *Journal of the Acoustical Society of America* 68, 1584–1599.
- Henry KS, Kale S, Scheidt RE, Heinz MG 2011 Auditory brainstem responses predict auditory nerve fiber thresholds and frequency selectivity in hearing impaired chinchillas. *Hearing research* 280, 236–244. [PubMed: 21699970]
- Janning MH, Whitworth CA, Rybak LP 1998 Experimental model of cisplatin ototoxicity in chinchillas. *Otolaryngology--Head and Neck Surgery* 119, 574–580. [PubMed: 9852528]
- Kim DO, Zahorik P, Carney LH, Bishop BB, Kuwada S 2015 Auditory Distance Coding in Rabbit Midbrain Neurons and Human Perception: Monaural Amplitude Modulation Depth as a Cue. *J Neurosci* 35, 5360–5372. [PubMed: 25834060]
- Konings A, Van Laer L, Van Camp G 2009 Genetic Studies on Noise-Induced Hearing Loss: A Review. *Ear Hearing* 30, 151–159. [PubMed: 19194285]
- Kujawa SG, Liberman MC 2006 Acceleration of age-related hearing loss by early noise exposure: evidence of a misspent youth. *Journal of Neuroscience* 26, 2115–2123. [PubMed: 16481444]
- Kujawa SG, Liberman MC 2009 Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J Neurosci* 29, 14077–85. [PubMed: 19906956]
- Lasky RE, Soto AA, Luck ML, Laughlin NK 1999 Otoacoustic emission, evoked potential, and behavioral auditory thresholds in the rhesus monkey (*Macaca mulatta*). *Hearing research* 136, 35–43. [PubMed: 10511622]
- Lobarinas E, Salvi R, Ding DL 2013 Insensitivity of the audiogram to carboplatin induced inner hair cell loss in chinchillas. *Hearing Research* 302, 113–120. [PubMed: 23566980]
- Luebke AE, Stagner BB, Martin GK, Lonsbury-Martin BL 2015 Influence of sound-conditioning on noise-induced susceptibility of distortion-product otoacoustic emissions. *J Acoust Soc Am* 138, 58–64. [PubMed: 26233006]
- McFadden SL, Henselman LW, Zheng X-Y 1999 Sex differences in auditory sensitivity of chinchillas before and after exposure to impulse noise. *Ear Hearing* 20, 164–174. [PubMed: 10229517]
- McFadden SL, Woo JM, Michalak N, Ding DL 2005 Dietary vitamin C supplementation reduces noise-induced hearing loss in guinea pigs. *Hearing Research* 202, 200–208. [PubMed: 15811712]
- Melcher JR, Guinan JJ Jr, Knudson IM, Kiang NY 1996 Generators of the brainstem auditory evoked potential in cat. II. Correlating lesion sites with waveform changes. *Hearing research* 93, 28–51. [PubMed: 8735067]
- Mhatre AN, Tajudeen B, Welt EM, Wartmann C, Long GR, Lalwani AK 2010 Temporary reduction of distortion product otoacoustic emissions (DPOAEs) immediately following auditory brainstem response (ABR). *Hearing Research* 269, 180–185. [PubMed: 20600743]
- Mills DM 2003 Differential responses to acoustic damage and furosemide in auditory brainstem and otoacoustic emission measures. *Journal of the Acoustical Society of America* 113, 914–924. [PubMed: 12597185]
- Milon B, Mitra S, Song Y, Margulies Z, Casserly R, Drake V, Mong JA, Depireux DA, Hertzano R 2018 The impact of biological sex on the response to noise and otoprotective therapies against acoustic injury in mice. *Biol Sex Differ* 9.
- Ngan EM, May BJ 2001 Relationship between the auditory brainstem response and auditory nerve thresholds in cats with hearing loss. *Hearing Research* 156, 44–52. [PubMed: 11377881]
- Ohlms LA, Lonsburymartin BL, Martin GK 1991 Acoustic-Distortion Products - Separation of Sensory from Neural Dysfunction in Sensorineural Hearing-Loss in Human-Beings and Rabbits. *Otolaryng Head Neck* 104, 159–174.

- Popelar J, Groh D, Mazelova J, Syka J 2003 Cochlear function in young and adult Fischer 344 rats. *Hearing Research* 186, 75–84. [PubMed: 14644461]
- Poulsen T, Han LA 2000 The binaural free field hearing threshold for pure tones from 125 Hz to 16 kHz. *Acustica* 86, 333–337.
- Probst R, Lonsburymartin BL, Martin GK 1991 A Review of Otoacoustic Emissions. *Journal of the Acoustical Society of America* 89, 2027–2067. [PubMed: 1860995]
- Rask-Andersen H, Ekvall L, Scholtz A, Schrott-Fischer A 2000 Structural/audiometric correlations in a human inner ear with noise-induced hearing loss. *Hearing research* 141, 129–139. [PubMed: 10713501]
- Salles FT, Andrade LR, Tanda S, Grati M.h., Plona KL, Gagnon LH, Johnson KR, Kachar B, Berryman MA 2014 CLIC5 stabilizes membrane-actin filament linkages at the base of hair cell stereocilia in a molecular complex with radixin, taperin, and myosin VI. *Cytoskeleton* 71, 61–78. [PubMed: 24285636]
- Shaffer LA, Withnell RH, Dhar S, Lilly DJ, Goodman SS, Harmon KM 2003 Sources and mechanisms of DPOAE generation: Implications for the prediction of auditory sensitivity. *Ear Hearing* 24, 367–379. [PubMed: 14534408]
- Shera C 2004 Mechanisms of mammalian otoacoustic emission and their implications for the clinical utility of otoacoustic emissions (vol 24, pg 86, 2004). *Ear Hearing* 25, 308–308.
- Stebbins WC, Hawkins JE Jr, Johnsson L-G, Moody DB 1979 Hearing thresholds with outer and inner hair cell loss. *American journal of otolaryngology* 1, 15–27. [PubMed: 95382]
- Stieve M, Mojallal H, Winter M, Battmer R, Lenarz T 2006 Normative auditory brainstem response data for hearing threshold in the rabbit. *Audiol Neuro-Otol* 11, 310–317.
- Strominger RN, Bohne BA, Harding GW 1995 Regenerated nerve fibers in the noise-damaged chinchilla cochlea are not efferent. *Hear Res* 92, 52–62. [PubMed: 8647746]
- Valero M, Burton J, Hauser S, Hackett T, Ramachandran R, Liberman M 2017 Noise-induced cochlear synaptopathy in rhesus monkeys (*Macaca mulatta*). *Hearing research* 353, 213–223. [PubMed: 28712672]
- Verhulst S, Jagadeesh A, Mauermann M, Ernst F 2016 Individual Differences in Auditory Brainstem Response Wave Characteristics: Relations to Different Aspects of Peripheral Hearing Loss. *Trends Hear* 20.
- Wada S-I, Starr A 1983 Generation of auditory brain stem responses (ABRs). III. Effects of lesions of the superior olive, lateral lemniscus and inferior colliculus on the ABR in guinea pig. *Electroencephalography and clinical neurophysiology* 56, 352–366. [PubMed: 6193949]
- Wang Y, Hirose K, Liberman MC 2002 Dynamics of noise-induced cellular injury and repair in the mouse cochlea. *J Assoc Res Otolaryngol* 3, 248–68. [PubMed: 12382101]
- Wightman FL, Kistler DJ 1992 The dominant role of low-frequency interaural time differences in sound localization. *J Acoust Soc Am* 91, 1648–61. [PubMed: 1564201]
- Zhou X, Jen PH-S, Seburn KL, Frankel WN, Zheng QY 2006 Auditory brainstem responses in 10 inbred strains of mice. *Brain research* 1091, 16–26. [PubMed: 16516865]

Highlights

- Octave-band noise at 133 dB SPL is sufficient to produce permanent threshold shifts.
- Threshold shifts are highly variable across individual ears.
- Female ears are more susceptible to noise overexposure than male ears.
- ABR and DPOAE threshold shifts predict total loss of outer hair cells.

Figure 1. Example rabbit ABRs and DPOAEs.

(A) Average click-evoked ABRs measured at the sound levels indicated on the left. Asterisk indicates threshold. (B) Average tone-evoked ABRs measured at 1 kHz, same as in A. Stimulus polarity was not alternated between repetitions at this frequency, and the cochlear microphonic (with 1-ms period) can be seen contaminating the signal at highest sound levels. Data in A and B from the same ear. (C) Magnitude spectrum of time-averaged acoustic waveform measured at the entrance of the ear canal during DPOAE measurement. Amplitude at primary (f_1) and secondary ($f_2 = 4$ kHz) tone frequencies and $2f_1-f_2$ DPOAE indicated. (D) $2f_1-f_2$ DPOAE amplitude (blue line) vs. secondary tone level (L_2) for the same ear and same f_1 and f_2 as in C. DPOAE threshold indicated by asterisk, where DPOAE amplitude crosses the criterion value (dashed line). Noise floor (red line).

Figure 2. ABR and DPOAE thresholds in unexposed rabbits.

(A) Click- and tone-evoked ABR thresholds (mean $\pm$ SD) (red line: males, $N = 14$ ears; blue line: females, $N = 39$ ears). For comparison: rabbit ABR thresholds from Borg and Engström (1983b) (brown dashed line), and rabbit behavioral thresholds from Borg and Engström (1983b) (magenta dashed line) and Heffner and Masterton (1980) (green dashed line). (B) DPOAE thresholds (mean $\pm$ SD) (red line: males, $N = 14$ ears; blue line: females, $N = 16$ ears). (C) Within-frequency, z-scored right- vs. left-ear ABR thresholds ($N = 182$). Unity line (black line). Pearson's correlation coefficient and p-value indicated in upper left corner. (D) Within-frequency, z-scored right- vs. left-ear DPOAE thresholds ($N = 60$), same as in C. (E) Within-ear-and-frequency, z-scored DPOAE vs. ABR thresholds ($N = 120$). Regression line (tan line).

Figure 3. Peak amplitudes and latencies of click-evoked ABR waveforms in unexposed rabbits. (A) Example average ABR waveforms from 4 rabbit ears in response to 70-dB SPL clicks. Roman numerals indicate wave number. (B) Peak amplitudes and (C) peak latencies (mean $\pm$ SD) of different wave numbers for male (red, N = 14) and female (blue, N = 39) ears. (D) Peak amplitude vs. peak latency for each wave number (N = 53 ears). Pearson's correlation coefficient and p-value indicated above each plot.

Figure 4. ABR threshold shifts following 60-min noise overexposure.

(**A**) Click- and tone-evoked ABR thresholds (mean $\pm$ SD) before (black solid line) and two weeks after (gray dashed line) noise overexposure ($N = 10$ ears). (**B**) Click- and tone-evoked ABR threshold shifts. Red dashes indicate medians. Data from same ear connected by same-colored line. Gray shaded regions in **A** and **B** indicate exposure band. DPOAE thresholds were not measured for this group.

Figure 5. ABR and DPOAE threshold shifts following 90-min noise overexposure. (A,B) Click-evoked and tone-evoked ABR threshold shifts for males and females, respectively (N = 10 ears each). (C,D) DPOAE threshold shifts for males (N = 10 ears) and females (N = 8 ears), respectively. In all panels: measured threshold shifts (open circles), minimum possible threshold shifts (solid squares), medians (red dashes). Data points from same ear connected by same-colored line.

Figure 6. Comparisons of within-frequency threshold shifts.

(A) Within-frequency ABR threshold shifts of right vs. left ears for males (red circles and crosses, $N = 20$) and females (blue circles and crosses, $N = 20$). (B). Same as in A for within-frequency DPOAE threshold shifts (males: $N = 20$, females: $N = 16$). (C) Within-ear, within-frequency threshold shifts of DPOAEs vs. ABRs (males: $N = 40$, females: $N = 32$). In all panels: data for which both threshold shifts in pair are measured threshold shifts (circles), data for which either one or both of threshold shifts in pair are minimum possible threshold shifts (crosses), unity line (black line).

Figure 7. Histopathology of noise-induced inner ear damage.

Cochlear whole-mount sections immunolabeled for Myo7A (green) and actin (red). Scale bar: 100 μ m. (A) Unexposed control. (B–D): Noise overexposed. White solid triangles point to intact OHCs, yellow solid triangles to intact IHCs, red solid triangles to support cells, dashed white triangles to holes left by missing OHCs, and dashed yellow triangle to missing IHCs.

Figure 8. Cochlear hair cell survival in unexposed cochleae.

Percent survival of OHCs (blue circles) and IHCs (magenta circles) within each cochlear frequency place. Medians (red dashes).

Figure 9. Cochlear hair cell survival in noise-overexposed cochleae.

(**A,B**) Percent OHC survival at each cochlear frequency place for females and males, respectively ($N = 6$ ears each), following 90-min noise overexposure. (**C,D**) Same as in **A** and **B**, but for percent IHC survival. In all panels: data from same ear connected by same-colored line, medians (red dashes), overexposure band (gray shaded area).

Figure 10. Relationship between IHC and OHC survival following noise overexposure. Two-dimensional histogram of the percent survival of IHCs and OHCs in the same ear at the same cochlear frequency location following 90-min noise overexposure (N = 72).

Figure 11. Relationship between threshold shifts and cochlear hair cell survival.

(A,B) Percent survival of OHCs vs. threshold shift for ABRs or DPOAEs ($N = 48$ each), respectively, following 90-min noise overexposure. Data were matched between tone frequency and cochlear frequency place, measured in the same ear. (C,D) Percent survival of IHCs vs. ABR or DPOAE threshold shifts, respectively, same as in A and B. In all panels: male data (red circles and crosses), female data (blue circles and crosses), measured threshold shifts (circles), minimum possible threshold shifts (crosses), critical threshold shifts (gray dashed lines).

Table 1.

Within-ear, pairwise-frequency correlation of ABR thresholds

kHz	0.5	1	2	4	8	16
0.5	—	0.68	0.62	0.46	0.31	0.30
1	0.68	—	0.57	0.27	0.25	0.23
2	0.62	0.57	—	0.59	0.51	0.35
4	0.46	0.27	0.59	—	0.71	0.46
8	0.31	0.25	0.51	0.71	—	0.61
16	0.30	0.23	0.35	0.46	0.61	—

Pearson's correlation coefficients (N = 53 ears). Bold values indicate significant correlation ($p < 0.05$).

Table 2.

Within-ear, pairwise-frequency correlation of DPOAE thresholds

kHz	2	4	8	16
2	—	0.54	0.01	0.40
4	0.54	—	0.24	0.40
8	0.01	0.24	—	0.41
16	0.40	0.40	0.41	—

Pearson's correlation coefficients (N = 30 ears). Bold values indicate significant correlation ($p < 0.05$).