Limitations of the Linearity of Stapes Displacement and Intracochlear Pressure in Response to Very High Level, Low Frequency Sounds

Running title: Stapes displacement and intracochlear sound pressure

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ABSTRACT

The stapes is suspended over the oval window membrane by the stapedial annular ligament (SAL), which restricts total peak-to-peak displacement of the stapes in response to loud sounds. Previous studies have suggested that for moderate (<130 dB SPL) sound levels intracochlear pressure (P_{IC}), measured at the base of the cochlea far from the basilar membrane, increases directly proportionally with stapes displacement (D_{Stap}), thus current models of middle ear function predict that peak IC sound pressure will vary linearly with D_{Stap} up to some saturation point. However, no direct tests of total D_{Stap} , nor of the relationship with P_{IC} during such motion, have been performed during acoustic stimulation of the human ear. In order to examine the relationship between D_{Stap} and P_{IC} to very high level sounds, measurements of D_{Stap} and P_{IC} were made in cadaveric human temporal bones. Specimens were prepared by mastoidectomy and extended facial recess to expose the ossicular chain. Sound pressure measurements were made in scala vestibuli (P_{SV}), scala tympani (P_{ST}), and external auditory canal (P_{EAC}), concurrently with laser Doppler vibrometry (LDV) measurements of stapes velocity (V_{Stap}). Stimuli were moderate (~ 100 dB SPL) to very high level (up to ~ 170 dB SPL), low frequency tones (20-2560 Hz). P_{IC} varied linearly with stapes displacement over a range of moderate level stimuli ($< \sim 150$ dB SPL), consistent with prior measurements at lower sound levels. With increasing sound pressure level in the ear canal both D_{Stap} and P_{SV} increased proportionally up to approximately ~ 150 dB SPL, above which both D_{Stap} and P_{SV} showed a distinct deviation from proportionality with P_{EAC} . D_{Stap} approached saturation at a value exceeding $150 \mu m$, which is substantially higher than the value reported for small mammals, while P_{SV} continued to rise at a lower rate. D_{Stap} magnitude was frequency dependent, with low frequencies showing larger displacements for a given P_{EAC} than higher frequencies. These results suggest that P_{IC} sound pressure is linearly dependent upon D_{Stap} at moderate but not high stimulation levels. Additionally, these results indicate that a current model of acoustic hazard, which was built using results from small animal testing (AHAH), underestimates the amount of energy entering the cochlea in response to high level sound stimulation, and should be revised.

Keywords Laser Doppler vibrometry, intracochlear pressure, high level sound, stapedial annular ligament

INTRODUCTION

The relationship between extreme acoustical events, such as blast waves produced by explosions, and damage to the auditory system has been under investigation since the development of high explosives in the 19th century (Sexton, 1887), and remains an issue of great importance, particularly in the modern military (Gee et al., 2013). The primary injuries of blast exposure include organs in the thoracic and abdominal cavities, the eyes and brain, and in particular, the ears (Mayorga, 1997). Injuries to the auditory system include tympanic membrane rupture and ossicular dislocation (Chandler et al., 1997; Mayo et al., 2006), as well as damaged and missing inner and outer hair cells in the cochlea (Patterson et al., 1997). Auditory injury has been used to predict injuries related to blast exposure, though previous research suggests that tympanic membrane rupture is a poor marker of pulmonary or intestinal injury (Leibovici et al., 1999). Pain, tinnitus, hyperacusis, vestibular dysfunction, and both temporary and permanent hearing loss, resulting from both conductive and sensorineural damage, are common following blast exposure (Fausti et al., 2009; Rao et al., 1980).

While the long term effects of blast injury in the auditory system have been well described (Fausti et al., 2009; Patterson et al., 1997), the precise dynamics of ossicular motion during high-level events remain unclear. In particular, responses during high level, low frequency stimulation, such as occur as a result of an explosion (especially out-doors where atmospheric attenuation will impact sound pressure propagation; Reed, 1977), have not been well characterized. One particularly important component affecting transmission of high level sound stimulation is the stapedial annular ligament (SAL), which suspends the stapes footplate over the oval window, and restricts the maximum displacement of the stapes (D_{Stap}) based upon the elasticity of the ligament fibers. Early experiments in cadaveric human temporal

bones suggested that the maximum displacement of the stapes footplate in response to static pressures was up to $100\mu\text{m}$ (summarized by Luciani, 1917), with specific estimates of stapes excursions of $70\mu\text{m}$ by Helmholtz and Politzer (Helmholtz, 1868; Politzer, 1864), $40\mu\text{m}$ by Bezold (Bezold, 1880), and $\sim 100\mu\text{m}$ by von Békésy (Von Békésy, 1960). More recent results from rabbit and cat suggested a peak-to-peak displacement limit of only $30\mu\text{m}$ (Guinan et al., 1967; Price, 1974; Yamamoto, 1953), and this value is a critical component in a current model of auditory hazard (the Auditory Hazard Assessment Algorithm for Humans (AHAH), available online) (AHAH 2015; Price, 2011; Price et al., 1991). A key insight from this model was that the SAL displacement limit acts to reduce low frequency energy transfer into the inner ear, thus a revised estimate of this limiting D_{Stap} in the human ear could have a substantial impact on the damage predictions of that model.

Recent work in cadaveric human temporal bones suggests that both D_{Stap} and intracochlear sound pressure (P_{IC}) increases roughly linearly with sound pressure level in the ear canal (P_{EAC}), as well as with one another, at moderate sound pressure levels ($< 140\text{ dB SPL}$), where D_{Stap} is not substantially limited by the characteristics of the ossicular chain (particularly the SAL) (Nakajima et al., 2009). Above this level, the motion of the ossicular chain becomes non-linear and shows substantial harmonic distortion (including subharmonics) at higher sound levels (Dallos et al., 1966; Guinan et al., 1967; Huang et al., 2012; Rosowski et al., 2007; Voss et al., 2000). It is unclear whether P_{IC} (and energy transfer into the cochlea) remains linearly related to P_{EAC} or to D_{Stap} at high sound pressure levels, particularly those where D_{Stap} shows saturation.

Here we test the hypothesis that P_{IC} varies linearly with D_{Stap} for sound pressure levels at which the SAL (or another component of the middle ear) limits D_{Stap} in cadaveric human temporal bones. Results provide the first estimates of the V_{Stap} and P_{IC} acoustic transfer functions for low

99 frequency (< 100 Hz) sounds for both (nearly) normal, and very high sound pressure
100 levels. Results go on to reveal the range of levels over which both D_{Stap} and P_{IC} increase linearly
101 with P_{EAC} , as well as values towards which the responses asymptote. Furthermore, the results
102 address the relationship between D_{Stap} and P_{IC} , and whether that relationship remains constant
103 with SPL. The implications for these results on auditory models, and injury prediction (i.e. the
104 AHAH model) are discussed.

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1. MATERIALS AND METHODS

The use of temporal bone tissue was in compliance with the University of Colorado Anschutz Medical Campus Institutional Biosafety Committee. Fifteen temporal bones with no history of middle ear disease (except presbycusis) were evaluated. Fresh-frozen hemi-cephalic specimens were obtained from cadavers undergoing autopsy with permission to use tissues and organs for research (Lone Tree Medical, Littleton, CO).

1.1. Temporal bone preparation

Prior to temporal bone preparation the specimens were thawed in warm water and the external ear canal and tympanic membrane were inspected for damage. The pinna and surrounding soft tissue were removed in order to improve access for the mastoidectomy. Similar to Tringali et al.(2010), temporal bones were prepared with a canal-wall-up mastoidectomy with extended facial recess, separated by a remaining buttress of bone, in order to visualize the body of the incus, the long-process of the incus, the stapes, and the round window, which were inspected to rule out damage and abnormalities. The facial canal was opened and the facial nerve removed immediately posterior to the oval and round windows to maximize exposure of the stapes and round window structures. The round window was inspected and false membrane removed, if present. Finally, the cochlear promontory near the oval and round windows was thinned in preparation for pressure probe insertion.

The hemi-cephalic specimens were fastened to a flat plate affixed via a dual-axis goniometer stage (Thorlabs, Inc., Newton, NJ) to a steel base plate. The specimen was positioned so that the pressure probes would enter the cochlea nearly vertically to prevent air insertion into the cochlea. Cochleostomies were created under a droplet of water using a fine pick. Fiber-optic pressure sensors (FOP-M260-ENCAP, FISO Inc., Quebec, Canada) were inserted through the

bony wall of the cochlea until just within the fluid ($\sim 100\mu\text{m}$) of the scala vestibuli (SV) and scala tympani (ST) using micromanipulators mounted on a horizontal bar affixed to the base plate. Sensors were sealed to the cochlea with alginate dental impression material (Jeltrate). Velocity of the stapes was measured with a single-axis laser Doppler vibrometer (LDV; HLC-1000 & CLV-700; Polytec Inc., Irvine, CA) positioned with a joystick-controlled aiming prism (HLVMM2) mounted to a dissecting microscope (Carl Zeiss AG, Oberkochen, Germany) parallel to the microscope's line of sight. The LDV was aimed at the stapes capitulum, and oriented at $\sim 45^\circ$ from the direction of the piston-like stapes motion. Microscopic (45-63 μm diameter) glass beads (P-RETRO-xxx, Polytec Inc., Irvine, CA) were placed on the stapes to ensure a strong LDV signal.

Following the conclusion of each experiment, the placement of both pressure probes was verified by carefully removing the bone between the two cochleostomies. The location and orientation of the basilar membrane was carefully assessed, and the compartment into which the probes projected was identified. Figure 1A shows an example preparation at the end of an experiment. Here, the pressure probes (P_{SV} & P_{ST}) have been removed, but their approximate positions during the experiment are indicated, and the small circular openings into the scala vestibuli and scala tympani are visible. The same specimen is shown in Figure 1B following bone removal. The probe insertion locations are indicated by the black circles, and the basilar membrane is clearly evident between the two. Visible in both photos are the stapes and stapes tendon (both are covered in glass microbeads which were used to boost LDV reflectance), as well as the incus long process, incudo-stapedial (I/S) joint, and round window membrane. Dashed ovals show the approximate locations of the round window and oval window (obscured by the stapes/stapes tendon; note that ovals do not reflect the actual size of these structures).

1.2. Sound presentation

All experiments were performed in a double-walled sound-attenuating chamber (IAC Inc., Bronx, NY). Sounds were generated digitally and presented to the specimen with a ~30 cm subwoofer (Morel UW 1258) driven by a 300W amplifier (Keiga KG5230), attached to a custom-built sound concentrating horn constructed of off-the-shelf PVC and copper components, their diameter tapering approximately exponentially from ~30 cm to ~1.25 cm over the course of ~12m. The sound presentation system was coupled to the ear with a section of silicone tubing (11 mm OD) tapered on one end to fit within the ear canal, stabilized with cyanoacrylate adhesive, and sealed with Jeltrate. The sound pressure level in the external ear canal was measured with both a probe-tube microphone (type 4182, Brüel & Kjær, Naerum, Denmark) and a fiber-optic pressure probe (FOP-M-BA, FISO, Inc.), due to the rated maximum of 164 dB SPL for the microphone, which were positioned approximately 1 cm from the tympanic membrane through small holes in the silicone tubing adapter, and sealed in place with Jeltrate. The frequency response of the sound delivery system was approximately flat from ~15 – 100 Hz, above which the level declined roughly linearly, decreasing by approximately 25 dB at 1 kHz. Stimuli were high level, low frequency tones, presented in 17 steps between 20 Hz - 5 kHz, with peak level exceeding 170 dB SPL below 100 Hz (the highest level tone produced was 173.4 dB SPL at 80 Hz). Sounds were presented and data were collected with an external sound card (Hammerfall Multiface II, RME, Haimhausen Germany), sampled at 44100 Hz, and controlled by a custom-built program in Matlab (Mathworks, Natick, MA).

1.3. Data Collection and Analysis

All acquired signals were band-pass filtered between 10Hz and 5kHz with a 2nd order Butterworth filter for data analysis. Signals were recorded and averaged across ten repetitions of each stimulus condition. Responses were quantified through Fourier analysis, where the response is quantified at the stimulus frequency. Velocity and pressure transfer functions were calculated in a manner consistent with a method described in an established standard (ASTM, 2014). Briefly, measured values of stapes velocity (V_{Stap}), as well as scala vestibuli (P_{SV}) and scala tympani (P_{ST}) sound pressures, are presented with respect to the sound pressure level in the external auditory canal (P_{EAC}). Corresponding transfer functions (H_{Stap} , H_{SV} , & H_{ST}) were computed from the responses of these measures to pure tone stimuli, using methods described previously (Devèze et al., 2013; Nakajima et al., 2009), e.g. equations 1-3:

$$H_{\text{Stap}} = V_{\text{Stap}} / P_{\text{EAC}} \quad (1)$$

$$H_{\text{SV}} = P_{\text{SV}} / P_{\text{EAC}} \quad (2)$$

$$H_{\text{ST}} = P_{\text{ST}} / P_{\text{EAC}} \quad (3)$$

Similarly, the differential intracochlear pressure transfer function was calculated as the complex difference between P_{SV} and P_{ST} , normalized to the sound pressure level in the external auditory canal (Nakajima et al. 2009):

$$H_{\text{Diff}} = (P_{\text{SV}} - P_{\text{ST}}) / P_{\text{EAC}} \quad (4)$$

The magnitude of the stapes velocity was adjusted using a cosine correction (Chien et al. 2006) based on an estimate of the included angle between the stapes primary axis (i.e. the direction of

piston-like motion) and the orientation of the LDV laser (typically $\sim 45^\circ$). Displacement was calculated in Matlab using a trapezoidal approximation to the integral of the LDV velocity signal. Responses are only shown for frequencies at which the signal-to-noise ratio was greater than 10dB, calculated by comparing measurements made immediately before and during sound presentation. Fiber optic pressure probes were factory calibrated, and the sensitivity verified by comparing sensor output normalized to the velocity (measured with the LDV) of a small cup of water driven by a Brüel & Kjær shaker (type 4810) across the range of frequencies tested. Sensor output was stable both within and across experiments, and no drift in sensor output was noted.

The sound pressure levels recorded in the ear canal near the tympanic membrane for each set of measurements, for the nine specimens meeting inclusion criteria, are shown in Figure 2. Inclusion criteria included: similarity of H_{Stap} to the 95% CI range reported previously (Rosowski et al. 2007), where responses consistently above this range suggest increased mobility of the ossicular chain (possibly due to damage), and responses consistently below this range suggest reduced mobility (potentially resulting from ossicular fixation due to otosclerosis); similarity of H_{SV} , H_{ST} , and particularly H_{Diff} to the range of responses reported previously (Nakajima et al. 2009), where deviations (particularly low H_{Diff}) suggest that pressure probe placement was incorrect; appropriate probe placement as verified via dissection (see above); lack of visible damage to the tympanic membrane or middle ear structures.

Sounds were digitally generated and attenuated down from the maximum. The output of the loudspeaker system is biased towards low frequencies, thus low-frequencies (< 100 Hz) tended to show the highest levels. Analysis is performed based on responses of each specimen to sound pressure levels grouped into three categories based on the sound pressure level of the sound stimulation at low (< 100 Hz) frequencies. In figures to follow, low level measurements,

i.e. the lowest level presented to each specimen (70 dB attenuation, or < 150 dB SPL at low frequencies), are shown with solid lines. Mid-level measurements (dashed) were those with low frequency sound presentation levels between 150 – 165 dB SPL (50 or 60 dB attenuation), and high-level measurements (dotted) were those above 165 dB SPL (30 or 40 dB attenuation). Responses were recorded to ten repetitions of each stimulus, presented in order from low to high frequencies (one presentation of each frequency per repetition), and from low to high levels (completing all repetitions of each level before increasing). Reducing the attenuation further resulted in substantial harmonic distortion of the sound produced, and constant or decreasing sound pressure level in the ear canal, thus the highest level assessed was close to 170 dB SPL in all specimens. In the following section, responses to each of these three levels will be assessed in both the time and frequency domains.

2. RESULTS

Velocity of the stapes capitulum, as well as sound pressure in the scala vestibuli and scala tympani near the oval and round windows are shown for nine temporal bones during presentation of moderate and high level, low frequency tones. Six specimens showed one or more transfer functions that deviated substantially from prior reports, thus were excluded from analysis. Two early specimens (160L, 55R) were not tested at all levels, thus do not appear in all figures. One additional specimen (292L) showed substantially lower H_{Stap} in the range of frequencies reported previously at the low level, but showed responses at lower frequencies and higher levels that were more consistent with prior reports (see Figure 7), thus are included in the analyses. The following analyses are roughly grouped based on the sound pressure level at which the lowest frequencies were presented, as shown in Figure 2 (i.e. the SPL of the 20 Hz presentation was: < 150 dB SPL for ‘Low’, < 170 dB SPL for ‘Mid’, and > 170 dB SPL for ‘High’).

2.1. Closed-field transfer functions

Figure 3 shows representative recordings from one example specimen (48L) in response to ten superposed repetitions of 30 Hz (Figure3A) and 80 Hz (Figure3B) tones at the **Low** sound pressure level (blue SPLs in Figure 2). The signal sent to the loudspeaker amplifier is shown at the top, followed by the superposed waveforms recorded from the microphone (green) and fiber-optic pressure probe (blue) in the ear canal near the TM (shown together to demonstrate the consistency across recording devices), the displacement of the stapes from the LDV, and the scala vestibuli (P_{SV}) and scala tympani (P_{ST}) sound pressures. Consistent with previous reports in human cadaver and small animals, motion of the stapes shows harmonic distortion at this level. This distortion is clearly visible at integer multiples of the fundamental frequency in the magnitude of the frequency spectrum (shown normalized to the maximum) in the right column, though all harmonics are ~20 dB below the fundamental in all signals (except perhaps D_{Stap}).

Stapes velocity (H_{Stap}), as well as scala vestibuli (H_{SV}), scala tympani (H_{ST}), and the differential ($H_{DIFF} = (P_{SV} - P_{ST})/P_{EAC}$) sound pressure transfer functions were computed as the ratio of each measure to the sound pressure level in the external auditory canal (EAC). Figure 4 shows measurements of H_{Stap} (A), H_{SV} (C), H_{ST} (D), and H_{DIFF} (B) magnitude at **Low** levels presented for seven of the nine temporal bones that met inclusion criteria. Recordings were stable over the course of ten repetitions, and are shown superimposed on the 95% confidence intervals/range of responses from prior reports (gray shading; Nakajima et al., 2009 for panels B-D; Rosowski et al., 2007 for panel A). In general, transfer function magnitudes in all four responses rose with frequency towards a peak near 1 kHz, and showed lower responses to lower frequency tones. Interestingly, the difference between H_{SV} and H_{ST} was smaller at lower (i.e. <100 Hz) than at

higher frequencies, and in a minority of specimens the absolute magnitudes reversed at the lowest frequencies, showing somewhat higher peak sound pressures in the scala tympani than scala vestibuli (e.g. specimen48R), similar to previous observations (Nakajima et al. 2015). Nevertheless, H_{DIFF} was generally consistent with the previous reports (Greene et al. 2015; Mattingly et al., 2015; Nakajima et al., 2009) in the range of frequencies tested previously.

Figure 5 shows transfer function phase of each response at the **Low** level, i.e. the phase of each signal with respect to the sound pressure level in the ear canal (P_{EAC}). Responses are superimposed on the 95% confidence intervals/range of responses from prior reports (Nakajima et al., 2009 for panels B-D; Rosowski et al., 2007 for panel A). H_{Stap} phase was stable at just under 90° with respect to the sound presentation for frequencies below approximately 200 Hz, and declined with higher frequency stimulation. Likewise, H_{SV} and H_{ST} phase were stable between $0-45^\circ$ for low frequencies that declined with higher frequency tones. H_{DIFF} phase was somewhat more variable across specimens, but was generally $\sim 90-180^\circ$ at low frequencies that decreased at higher frequencies, consistent with prior reports (Nakajima et al. 2009). All four specimens showed a general correspondence with the range of responses reported previously.

Figure 6 shows representative waveforms of 30 and 80 Hz tones presented at the **Mid** level (black SPLs in Figure 2) for the same specimen as in Figure 3. In contrast to waveforms at the **Low** SPLs, which closely resemble P_{EAC} , these response waveforms show substantial harmonic distortion that is either not present, or much lower in magnitude, in the ear canal sound pressure waveform. Nevertheless, P_{SV} and P_{ST} waveforms appear to remain consistent with one another, as well as with D_{Stap} . Interestingly, the even numbered harmonics (2f, 4f, etc.) are less prominent than the odd harmonics (3f, 5f, etc.) in all signals, and are lower in magnitude relative to the fundamental in the stapes displacement than in the intracochlear pressure measurements.

Importantly, although the second harmonic is visible in the response of the EAC pressure probe, it remains ~20 dB lower in magnitude than the fundamental frequency of the response. Also note that for *Mid* and *High* SPL conditions the microphone signal is limited by the power supply, resulting in a clipped signal at high pressure magnitudes, thus the EAC pressure probe is used rather than the microphone for these conditions, and the microphone signal is not shown.

Transfer function magnitudes calculated for responses to the *Mid*SPLs are shown in Figure 7. Responses are once again superimposed onto the 95% CI range (gray bands) of responses observed previously (Nakajima et al., 2009 for panels B-D; Rosowski et al., 2007 for panel A). Transfer function magnitudes are not directly affected by the harmonic distortion observed in Figure 6, since the transfer function at each frequency is computed from the magnitude at the fundamental frequency only. Similar to the *Low*SPL condition, responses in H_{Stap} , H_{SV} , and H_{ST} are comparable to those prior reports over the range of frequencies tested; however, H_{DIFF} shows a somewhat lower magnitude in most specimens compared to some (e.g.: Nakajima et al., 2009), but not all (Greene et al. 2015; Mattingly et al., 2015) prior reports. Similarly, all responses show somewhat reduced magnitudes compared to the *Low* stimulation level (Figure 4), particularly for low frequency tones – that is, intracochlear pressure increases at a slower rate than sound pressure level at these levels.

In order to explore this observed magnitude decrease with stimulus level further, the mean ($\pm$ SEM) transfer function magnitudes (computed on the real component of the transfer function, in the log domain) across the population of specimens are shown in Figure 8 for *Low*(blue), *Mid*(black), and *High*(red) SPL conditions. In general, transfer function magnitudes showed similar trends with frequency across levels, with magnitudes showing similar peaks in magnitude around 1 kHz. However, the overall magnitude decreased with stimulus level, predominantly for

low frequency stimulation, for all four measures (except for V_{Stap} between ~150-1500 Hz, where a small increase is visible between the *Low* and *Mid* conditions). In particular, transfer function magnitudes to low frequencies ($< 100\text{ Hz}$), where the sound level was greatest, decreased by ~15 dB in all four responses between the *Low* ($< 150\text{ dB SPL}$) and *High* level ($> 170\text{ dB SPL}$) SPL conditions. Transfer function magnitudes also decreased at higher frequencies for *High* level stimulation, but to a lower degree ($< 10\text{ dB}$ for $> 500\text{ Hz}$ tones), and the distributions of responses tended to overlap substantially at these higher frequencies.

The changes observed across sound pressure levels were assessed with a one-way ANOVA with sound pressure level group (i.e. *Low*, *Mid*, or *High*) as the independent, and transfer function magnitude as the dependent variables. The ANOVA indicated significant main effects for all four measures (H_{Stap} : $F_{2,290} = 3.85$, $p = 0.0224$; H_{SV} : $F_{2,293} = 21.34$, $p = 2 \times 10^{-9}$; H_{ST} : $F_{2,280} = 34.42$, $p = 4 \times 10^{-14}$; H_{Diff} : $F_{2,278} = 16.12$, $p = 2 \times 10^{-7}$). Post hoc testing with a Tukey's HSD test reveals that *Low* and *High* levels are significantly different for H_{Stap} , and all three levels are significantly different from each other for H_{SV} , H_{ST} , and H_{Diff} . These reductions in transfer function magnitudes suggest that the limitation of the response at maximum excursion reduces the magnitude of the fundamental frequency of the response at *Mid* and *High* SPLs concurrently with the harmonic distortion observed in Figure 6. The frequency and level dependence of this effect will be explored further in the next section.

Figure 9 shows the mean ($\pm \text{SEM}$) transfer function phases calculated for the *Low*, *Mid*, and *High* SPL conditions. Transfer function phases were largely unaffected by the level for low frequencies (i.e. $< \sim 300\text{ Hz}$), except in H_{DIFF} , which shows a shift in mean phase from $\sim 135^\circ$ for *Low* and *Mid*, and $\sim 45^\circ$ for *High* SPL stimulation. At higher frequencies, the rapid decrease in phase appears to have shifted from $\sim 1000\text{ Hz}$ down to $\sim 600\text{ Hz}$ in H_{SV} and H_{ST} , and may be

decreasing more rapidly than has been reported at lower levels (Nakajima et al. 2009; gray bands).

The changes observed across sound pressure levels were assessed with a one-way ANOVA with sound pressure level group (i.e. *Low*, *Mid*, or *High*) as the independent, and transfer function phase as the dependent variables. The ANOVA indicated significant main effects for the three pressures, but not H_{Stap} (H_{Stap} : $F_{2,290} = 2.18$, $p = 0.115$; H_{SV} : $F_{2,293} = 9.07$, $p = 1.5 \times 10^{-4}$; H_{ST} : $F_{2,280} = 5.11$, $p = 0.0066$; H_{Diff} : $F_{2,263} = 6.06$, $p = 0.0027$). Post hoc testing with a Tukey's HSD test reveals that the *Low*SPL condition is significantly different than both *Mid*, and *High* levels for H_{SV} and H_{ST} , whereas only *Low* and *High* levels are significantly different for H_{Diff} .

2.2. Linearity of Stapes Displacement and Scala Vestibuli Pressure with SPL

Figure 10A shows the peak-to-peak D_{Stap} amplitude, measured in each trial recorded in one specimen (48L), as a function of sound presentation level (recorded level in dB SPL on the abscissa, level group from Figure 2 in corresponding colors). Responses are shown for a set of preliminary recordings made with a small closed-field loudspeaker (TDT MF1) (blue), as well as for *Mid* (black), and *High* (red) SPL stimulation. Values are shown for frequencies less than 900 Hz (except for responses from the small loudspeaker, for which responses are shown from 100 Hz to 10 kHz stimulation) to highlight responses to the highest level sounds recorded. Responses are superimposed over a 20 dB/decade line indicating a linear relationship (Guinan and Peake 1967). Similar to those previous results in the cat, D_{Stap} increased proportionally with sound pressure level up to ~150 dB SPL, above which displacement deviates from the 20 dB/decade line.

Intracochlear sound pressure level was similarly assessed by plotting the peak-to-peak P_{SV} amplitude as a function of P_{EAC} SPL, with the axes transposed, in Figure 10B. Responses are shown for the same conditions as for the displacement measurements presented at left, and are shown superimposed on an arbitrarily drawn 20 dB/decade line. P_{SV} rises linearly with increasing P_{EAC} SPL up to ~150 dB SPL, above which responses begin to saturate, showing compression relative to P_{EAC} , similar to D_{Stap} .

The relationship between D_{Stap} and P_{SV} is assessed in Figure 10C. Straight lines are drawn at locations corresponding to the straight lines in the left and bottom plots (black), as well as at 10 dB (gray) and 20 dB (dashed gray) higher P_{SV} for reference. Consistent with a proportional dependence of P_{SV} on D_{Stap} , the responses generally fall along a single straight line across the range of displacements/pressures observed. In particular, while both D_{Stap} and P_{SV} show a departure from proportionality at ear canal sound pressure levels above ~150 dB SPL, this nonlinearity appears comparable in magnitude in the two measures, thus the relationship between D_{Stap} and P_{SV} remains consistent even at the highest levels tested (*High* SPLs, red symbols).

2.3. Stapes Displacement and Scala Vestibuli Pressure as a function of SPL and frequency

The above plots of velocity and pressure as a function of P_{EAC} SPL reveal a general trend away from proportionality for levels greater than ~150 dB SPL; however, response transfer functions reveal a clear frequency dependence of the response magnitude. In order to illustrate this joint dependence on level and frequency more directly, the peak-to-peak D_{Stap} and P_{SV} from one specimen (160L) are shown as a function of P_{EAC} SPL in Figures 11A & B, respectively.

Here, lines connect the mean (standard deviation is smaller than the marker size) responses assessed at the *Mid* and *High* SPL conditions for each frequency, where both color and line width indicate stimulus frequency. The black lines represent the 20 dB per decade lines shown in Fig 10A-B. Two main points can be assessed from these plots (and are described in the following paragraphs): the maximum responses (D_{Stap} and P_{SV}) observed, and the SPL at which these responses saturate.

First, since both D_{Stap} and P_{SV} saturate at high P_{EAC} SPLs, the asymptote of each response can be predicted based upon the maximum observed stimulus magnitude. Mean D_{Stap} maximum ($\pm$ standard deviation) across the nine specimens was $133.0 \pm 36.3 \mu\text{m}$, which is substantially higher than prior estimates from small animals (e.g. $\sim 20 \mu\text{m}$ in the cat; $\sim 30 \mu\text{m}$ in rabbit) (Guinan et al., 1967; Yamamoto, 1953). Mean P_{SV} maximum was more variable across the nine specimens tested at $12.16 \pm 9.03 \text{ kPa}$, and generally did not approach a clearly defined asymptote, thus maximum pressures may continue to rise with increasing P_{EAC} SPL. Note that higher frequency responses tended to show lower amplitudes than low frequency responses at a given SPL, thus the maximum values reported here may not hold for higher frequencies.

Second, the P_{EAC} SPLs at which D_{Stap} and P_{SV} saturate cannot be measured directly due to the frequency dependence of the response magnitudes; however, they can be estimated by assessing the rate of change in response amplitude with level, since these responses should rise linearly with SPL for low levels, but should saturate and show no increase at higher levels. The responses of each specimen were sampled relatively sparsely in level, thus a direct measurement of the asymptote value for each frequency is not possible. An estimate of the level at which these responses asymptote was calculated for each specimen by comparing the slopes of the response at each frequency to the expected slopes (20 dB/dec.) shown in Figure 10A-B. Of note, this

analysis assumes frequency independence over the range of frequencies tested. Qualitatively, the slopes of these straight lines for D_{Stap} and P_{SV} shown in figure 11 are comparable to the 20 dB per decade lines from Figure 10A-B for P_{EAC} SPLs $< \sim 150$ dB SPL, and, consistent with a response asymptote, line slopes approached zero when either the *Mid* or *High* SPL was above ~ 150 dB SPL.

In order to quantify this observation, slopes are shown in Figure 12 as a function of the *High* SPL. Based on values reported for the cat, D_{Stap} increases linearly with sound level below ~ 130 dB SPL, thus we can reasonably expect slopes to approach 20 dB/decade for low levels. Similarly, for higher levels, the mechanical characteristics of the SAL will limit stapes motion, such that peak-to-peak D_{Stap} will approach a single value (assuming the level does not damage these structures), thus the slope should approach zero at high levels. Slopes appear to approach zero in a roughly sigmoidal fashion for both D_{Stap} and P_{SV} , thus in order to aid in quantification, each trend was quantified by fitting a four-parameter logistic function to the slopes of each specimen (black lines) using the L4P.m function available on the Matlab Central File Exchange (Cardillo, 2012).

D_{Stap} slopes as a function of stimulus level are shown in Figure 13A, and consistent with these predictions, demonstrate a decrease from near 20 dB/decade towards zero starting at ~ 140 dB SPL in all specimens. The inflection points of the curve fits provide a convenient measure of the sound pressure at which the response deviates from linearity with P_{EAC} , averaging 159.9 ± 8.2 dB SPL across specimens. Additional fit parameters include (mean $\pm$ standard deviation): the lower asymptote (22.6 ± 1.4 dB/decade), which is close to the 20 dB/decade value as expected; Hill's slope, a measure of curve steepness (423 ± 151 dB/decade/dB SPL), and upper

asymptote(3.36 ± 1.34 dB/decade), which approaches zero. Logistic functions were well-fit to the data (mean $R^2 = 0.82 \pm 0.24$, RMSE = 0.16 ± 0.13).

P_{SV} slopes as a function of the higher stimulus level tested are analyzed similarly in Figure 12B. P_{SV} slopes are more variable across specimens than D_{Stap} , but show a similar trend to decline from ~ 20 dB/decade towards zero at higher levels. Logistic functions were less well fit to the data (mean $R^2 = 0.27 \pm 0.36$, RMSE = 0.14 ± 0.06), and predict a mean ($\pm$ standard deviation) Hill's slope of 264 ± 230 dB/decade/dB SPL and inflection point of 145.2 ± 57.9 dB SPL. The lower Hill's slopes indicate that peak to peak P_{SV} approaches an asymptote with level more slowly than D_{Stap} .

3. DISCUSSION

Recent technological advances have allowed direct measurement of intracochlear pressure in response to sound stimulation in intact human temporal bones (Greene et al. 2015; Mattingly et al., 2015; Nakajima et al., 2009; Olson, 1998; Olson, 1999). In this report we set out to characterize ossicular motion and intracochlear pressure in response to low frequency and high level sound stimulation. These data provide insight into the relationship between stapes motion and intracochlear pressure, and provide a quantitative assessment of assumptions and predictions in a modern acoustic hazard model.

3.1. Low-frequency response characterization

Previous measures of stapes velocity and intracochlear sound pressure responses were recorded at intermediate sound frequencies (i.e. 100 Hz to 10 kHz) due to equipment and measurement limitations, and since those frequencies tend to contain the majority of the

information from speech and other behaviorally relevant stimuli (e.g. Nakajima et al., 2009; Rosowski et al., 2007). The ability of our sound presentation system to generate high level sounds with acoustic energy below 100 Hz allows us to establish, for the first time, a baseline for responses at these very low-frequency sounds. Additional motivation is provided by the observation that low frequency energy is often concentrated in many impulsive noise and blast waveform recordings in the free-field (Reed, 1977).

Velocity and pressure transfer functions (H_{Stap} , H_{SV} , and H_{ST}) were generally in agreement with previous reports for frequencies above 100 Hz (Nakajima et al., 2009; Rosowski et al., 2007). In particular, harmonic distortion was observed in the motion of the stapes for mid and high level stimuli (Guinan et al., 1967; Rosowski et al., 2007; Voss et al., 2000), and peaks in H_{Stap} and H_{SV} are clearly evident for frequencies around 1 kHz, in addition to a 10-20 dB difference between H_{SV} and H_{ST} in this frequency range (Nakajima et al., 2009; Rosowski et al., 2007). A trend of H_{Stap} and H_{SV} toward lower gain with decreasing frequency is visible in prior measurements (Nakajima et al., 2009), and appears to continue along the same trajectory for frequencies below 100 Hz. Values of H_{SV} , which typically peak near 1 kHz (e.g. Figure 4) fall off at low frequencies to the point that H_{ST} appears similar, or shows higher gain (re: P_{EAC}) in some specimens below 100 Hz. A similar reversal of relative intracochlear pressures at frequencies below 400 Hz was observed in one specimen and described in Nakajima et al. (see Figure 2 ear 039; 2009), and the cause remains unclear. The similarity in scala vestibuli and scala tympani sound pressures in many specimens, however, may suggest the presence of a substantial bone-conducted component of the sound transmission to the inner ear, resulting from shaking of the sound booth/table/specimen mount and cadaver specimen by the sound source, particularly at the lowest frequencies. Nevertheless, the data recorded at the lowest levels tested here are generally

consistent with prior reports, and extend baseline measurements of stapes velocity, and intracochlear pressures to the low-frequency limit of human hearing (generally considered to be ~20 Hz), albeit for high sound pressure levels (~130 dB SPL).

3.2. The relationship between intracochlear pressure and stapes displacement

The relationship between intracochlear pressure and ossicular motion has not been thoroughly examined, but since the stapes footplate provides the input to the scala vestibuli via the oval window, and since the two show similar dependence on sound pressure level in the ear canal at moderate levels, it is natural to assume a direct, positively correlated relationship between the two. No direct comparison of these measures has been reported, but indirect evidence is found in the reports of Nakajima et al. (2009), who reported a linear relationship between P_{IC} and P_{EAC} for tones presented between 80-130 dB SPL, and Aibara et al. (2001) who reported V_{Stap} for sound presentation between 60-120 dB SPL with no mention of level effects. Since both V_{Stap} and P_{SV} are apparently linearly related with P_{EAC} in these reports (Nakajima et al., 2009), one can thus assume that the two are linearly related in the range of sound pressure levels tested (60-130 dB SPL).

Rather than comparing D_{Stap} and P_{SV} explicitly, prior reports have investigated the cochlear input impedance, defined as: $Z_C = P_{SV} / (V_{Stap} * A_{FP})$, where A_{FP} is the area of the stapes footplate, which is typically assumed to be 3.2 mm^2 (Nakajima et al., 2009; Von Békésy, 1960). Those prior studies report Z_C that is relatively flat across frequency in the range of 10-100 $G\Omega$, and which is consistent across levels (although level dependence does not appear to have been thoroughly assessed in these reports). Figure 13 shows the mean ($\pm$ SEM) Z_C (assuming $A_{FP} = 3.2 \text{ mm}^2$) across the population of specimens included in the current study, at the *Low*, *Mid*, and

Highlevel stimulation conditions. Responses are superimposed onto lines representing the mean responses observed in three prior reports (Merchant et al., 1996; Nakajima et al., 2009; Aibara et al. 2001). Results are assessed with a two-way ANOVA with Z_C as the dependent variable, revealing a significant main effect of frequency ($F_{14,189} = 15.7$, $p \ll 0.01$), revealed by the higher Z_C at the lowest frequencies (i.e. < 80 Hz) via a post-hoc Tukey HSD test; neither level ($F_{2,189} = 0$, $p = 1$), nor the interaction term ($F_{28,189} = 0$, $p = 1$), were significant.

The similarity between these responses and those prior reports (and across stimulus level) suggests that the proposed relationship between D_{Stap} and P_{SV} is consistent even at levels at which substantial distortion from a saturating nonlinearity appears in both responses. Distortion in both V_{Stap} and P_{SV} signals arises for high level stimulation in the form of harmonic distortion. Substantial amplitude is observed at integer multiples of the fundamental frequency, with more prominent odd- than even-numbered harmonics.

Subharmonic distortion is a well characterized feature of middle mammalian middle ear responses. It has been observed in EAC SPL measurements for loud tonal stimulation (> 140 dB SPL in humans, 95 dB SPL in guinea pigs) (von Gierke 1950), is perceived by humans for 140 dB SPL tone presentation (Eldredge 1951), and has been reported in intracochlear pressure measurements in gerbils (Huang et al. 2012). This distortion is hypothesized to originate from asymmetric stiffness in the tympanic membrane (Pong and Marcaccio 1963), and not an active middle ear process, as subharmonics were observed in pig temporal bones (Usanov et al. 2007). Interestingly, no substantial subharmonics were observed in the current measurements, potentially suggesting that the generation of subharmonics is a largely high-frequency phenomenon.

3.3. Acoustic hazard model validation

The AHAH model explicitly models each component of the acoustic transmission chain into the cochlea with equivalent electrical components. The results presented in this report confirm that intracochlear sound pressure is directly proportional to stapes displacement for low to moderate (i.e. up to ~155 dB SPL) sound levels. This suggests that the AHAH model of acoustic hazard, used to predict auditory injury during impulsive noise exposure (Price et al., 1991), faithfully represents energy transfer from the middle- into the inner-ear for these sound levels, but may need revision in order to accurately predict injury at higher levels. In particular, these data reveal a substantial difference between the maximum peak-to-peak D_{stap} predicted by the model, and that observed in cadaveric human temporal bones.

Maximum peak-to-peak stapes displacement simulated in the AHAH model was set to a value of 40 μm based upon measurements made in cat and rabbit (Guinan et al., 1967; Price, 1974; Yamamoto, 1953). This value is substantially lower than has been reported by several investigators in cadaveric human specimens when applying a static pressure to the oval window (Bezold, 1880; Helmholtz, 1868; Politzer, 1864; Von Békésy, 1960). Similarly, a more recent investigation of the mechanical properties of the stapedial annular ligament reported force-displacement curves consistent with a maximum displacement of approximately 100 μm (Gan et al., 2011). The current results suggest that the stapes can displace more than 150 μm peak to peak at very high sound pressure levels. The AHAH model, which assumes an order of magnitude smaller displacement limit, therefore, appears to underestimate the energy transferred to the cochlea, thus likely underestimates the injury caused by a loud sound exposure. Future work is required to modify the model to better predict this energy propagation, and better

determine the frequency dependence of these effects (since these results are primarily low-frequency).

3.4. Limitations to the current study

Stapes motion as reported in this study is taken from a velocity measurement at one point on the stapes. In consequence, stapedia velocity and quantities derived from it, including stapes displacement, cochlear volume velocity, and cochlear impedance, are derived from this single-point measurement. This simplification omits two aspects of ossicular mechanics which may, when introduced, significantly alter our conclusions.

First, a number of studies have shown that three-dimensional stapedia motion is quite complex, combining simple piston-like motion with rocking motion in more than one dimension, and that the relative magnitudes of these components vary greatly over the normal range of auditory frequencies and sound levels. For this reason, single-point measurement may fail to capture some ossicular dynamics important to the relationship between stapedia velocity and intracochlear pressure. Of note, studies of the three-dimensional stapedia motion (for example, Hato et al. 2003, Sim et al. 2010) have found that the rocking component(s) becomes substantially more important at higher frequencies; for this reason, we feel that the single-point approximation is reasonable at the lower frequencies tested in this report. Nevertheless, this study was intended to explore stapedia velocities and intracochlear pressures for frequencies below, and sound pressure levels above, those typically measured. For exactly this reason, the possibility cannot be excluded that three-dimensional stapes motion at these frequencies and sound pressure levels differ significantly from motion within the normal range, affecting our conclusions for the reasons discussed above.

Second, our analysis treats the apparent limitation of stapedial motion as a consequence of nonlinear stiffness in the stapedial annular ligament. It is also possible that compliance in the incudomalleolar joint (or some other component of the middle ear) contributes significantly to a reduction in stapedial motion, and that this compliance may itself be nonlinear, taking up some displacement that might otherwise be transmitted to the cochlear fluid at high sound pressure levels. One recent report (Gerig et al. 2015) found that IM joint mobility is not a major contributor to ossicular chain compliance at frequencies below 2 kHz. We do not discount the possible contribution of the IM joint (or other components of the ossicular chain) to the nonlinearity observed in this study, but believe it to be of secondary importance at very low frequencies.

4. CONCLUSIONS

The current results generally agree with the results from prior reports suggesting that P_{SV} is directly proportional to D_{Stap} for levels both below and above which harmonic distortion appears due to a saturating nonlinearity. These results suggest that the AHAAH model should accurately predict acoustic injury for sounds below this level; however, peak-to-peak D_{Stap} is substantially larger than predicted by this model, and the lower than predicted deviation from proportionality of P_{SV} at high sound levels suggest that the assumption of a linear relationship between P_{IC} and D_{Stap} at high levels ($> \sim 150$ dB SPL) must be reassessed. Finally, these results extend our knowledge of the responses of healthy temporal bones above this level (up to ~ 170 dB SPL), and provide baseline (< 150 dB SPL) data for low frequencies down to the low frequency limit of human hearing (~ 20 Hz).

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592

FIGURE LEGENDS

Fig. 1: Photomicrographs showing the placement of the intracochlear pressure probes in the scala vestibuli and scala tympani before (A), and after (B) dissection away the bone between the two cochleostomies in specimen 55R (right ear). Dashed circles indicate the approximate locations of the oval and round windows (not to scale); solid circles indicate the entry points of the pressure probes into the cochlea. Note the glass beads located on the stapes capitulum and stapes tendon (A), and the location and orientation of the basilar membrane (B).

Fig. 2: Sound pressure levels in the ear canal during each set of recordings. Specimen number is indicated by marker symbol and line color. Sets of recordings with roughly comparable levels are grouped by color (identified by the level at 20 Hz: *Low*: blue: ≤ 150 dB SPL; *Mid*: black: > 150 dB SPL & ≤ 170 dB SPL; *High*: red: > 170 dB SPL).

Fig. 3: Examples of the stimulus waveform (signal), sound pressure in the external auditory canal near the tympanic membrane recorded with a fiber-optic pressure probe (blue; EAC) and probe-tube microphone (green, Mic), stapes displacement (D_{Stap}), and intracochlear pressures measured in the scala vestibuli (P_{SV}) and scala tympani (P_{ST}), recorded during presentation of 30 Hz (A) and 80 Hz (B) tones presented at *Low* sound pressure levels in specimen 48L. The FFT of each signal is shown on right normalized to height of the FFT band at the stimulus frequency.

Fig. 4: (A) Stapes velocity, (B) differential, (C) scala vestibuli and (D) scala tympani sound pressure transfer function magnitudes recorded at *Low* SPLs. Responses correspond to blue lines and markers shown in Figure 2.

616

617 Fig. 5: (A) Stapes velocity, (B) differential, (C) scala vestibuli and (D) scala tympani sound
 618 pressure transfer function phases recorded at the *Low* SPLs. Responses correspond to blue lines
 619 and markers shown in Figure 2.

620

621 Fig. 6: Examples of the stimulus waveform (signal), sound pressure in the external auditory canal
 622 near the tympanic membrane recorded with a fiber-optic pressure probe (blue; EAC) and probe-
 623 tube microphone (green, Mic), stapes displacement (D_{Stap}), and intracochlear pressures measured
 624 in the scala vestibuli (P_{SV}) and scala tympani (P_{ST}), recorded during presentation of a 30 Hz (A)
 625 and 80 Hz (b) tone presented at the *Mid*SPL in specimen 48L. The FFT of each signal is shown
 626 at right, normalized to height of the FFT band at the stimulus frequency.

627

628 Fig. 7: (A) Stapes velocity, (B) differential, (C) scala vestibuli and (D) scala tympani sound
 629 pressure transfer function magnitudes recorded at *Mid* SPLs. Responses correspond to black
 630 lines and markers shown in Figure 2.

631

632 Fig. 8: Comparison of transfer function magnitudes across sound pressure levels, presented for
 633 (A) stapes velocity, (B) differential, (C) scala vestibuli and (D) scala tympani. Solid lines
 634 and gray areas represent the mean and SEM range, respectively, for TF magnitude across
 635 specimens.

636

Fig.9: Comparison of transfer function phases across sound pressure levels, presented for (A) stapes velocity, (B) differential, (C) scala vestibuli and (D) scala tympani. Solid lines and gray areas represent the mean and $\pm$ SEM range, respectively, for TF phase across specimens.

Fig.10: Peak-to-peak stapes displacement (A) and scala vestibuli pressure (B) as a function of sound pressure level, and compared to one another (C), for one specimen 48L, across the three levels tested. Note that the blue points represent preliminary measurements made with a different loudspeaker, and do not directly correspond to the *Low* measurements, thus are labeled ‘baseline’ here. Diagonal lines represent: in D_{Stap} , the 20 dB per decade line after Guinan and Peake (1967); in P_{SV} , an arbitrary 20 dB per decade line; and in the comparison, the union of diagonals in the D_{Stap} and P_{SV} plots (black), as well as lines shifted towards higher pressures by 10 (gray solid) and 20 dB (gray dashed).

Fig.11: Mean of (A) D_{Stap} , and (B) P_{SV} , for each frequency presented, as a function of EAC SPL for one specimen, 160L. Frequency is represented by color and line thickness. Responses are shown for the two lower levels presented (i.e. low, or $\leq \sim 155$ dB SPL, and the mid level, $\leq \sim 165$ dB SPL).

Fig.12: Slopes of the straight lines observed in (A) D_{Stap} , and (B) P_{SV} (with axes reversed) vs SPL curves shown in Figure 11 shown as a function of P_{EAC} SPL. Values are shown as a function of the higher sound pressure level presented. Color and symbol indicate specimens. Points represent individual frequencies tested. Horizontal black lines indicate 20 dB per decade.

660 Fig.13: Mean ($\pm$ SEM) cochlear input impedance (Z_C) as a function of frequency for the three
661 levels tested. Responses are superimposed onto the mean Z_C shown in three prior reports (Aibara
662 et al. 2001, Merchant et al. 1996, Nakajima et al. 2009).

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763

Figure 1

A

B

Figure 2

Figure 3

A

Time Domain

Frequency Domain

B

Time re: stimulus onset (seconds)

Frequency (Hz)

Figure 4

Figure 5

Figure 6

A**Time Domain** **Frequency Domain** **B**

Time re: stimulus onset (seconds)

Frequency (Hz)

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

A **B**

Figure 12

A **B**

Figure 13