Nonlinear vibration response measured at umbo and stapes in the rabbit middle ear

Abstract

Using laser vibrometry and a stimulation and signal analysis method based on multisines, we have measured the response and the nonlinearities in the vibration of the rabbit middle ear at the level of the umbo and the stapes. With our method we were able to detect and quantify nonlinearities starting at sound pressure levels of 93 dB SPL. Further research will be needed to pinpoint the source of these nonlinearities, but the current results show that no significant additional nonlinearity is generated as the vibration signal is passed on through the middle ear chain.

Nonlinearities are most prominent in the lower frequencies (125 Hz to 1 kHz), where their level is about 40 dB below the vibration response at an input of 120 dB SPL. The level of nonlinearities rises with a factor of nearly 2 as a function of sound pressure level, indicating that nonlinear distortions may become important at very high sound pressure levels, such as are used in high-power hearing aids.

1. Introduction

Nonlinearity in the mammalian middle ear has been investigated by several authors and using a variety of techniques. The purpose of most of these studies was to exclude the influence of middle ear nonlinearity on other measurements. One of the earliest attempts to measure nonlinearity was made by Guinan and Peake (1967). They measured the motion of the stapes in cats and reported that it behaves linearly up to around 130 dB SPL for frequencies below 1500 Hz, and 140 to 150 dB SPL for higher frequencies. More than a decade later, Nedzelnitsky (1980) measured intracochlear pressures in cats and found them to be linearly related to sound pressure at the eardrum up to 140 dB SPL. Measurements in human temporal bones were performed by Goode et al. (1994). They used laser Doppler vibrometry to measure both the umbo and the stapes and observed no signs of nonlinearity at sound pressure levels below 124 dB. In 2000, Voss et al. also measured stapes velocity and found no signs of nonlinearity below 130 dB SPL. Given these results, the transfer function of the middle ear was assumed to be completely linear up to 130 dB SPL.

In 2010 a new, very sensitive method based on multisine excitations was developed and was used to measure the middle ear of gerbils (Aerts and Dirckx, 2010). These new measurements detected small nonlinear distortions appearing above the noise at around 96 dB SPL. However, these measurements only examined the vibration response of the umbo, and did not comment on the effects of transmission through the ossicular chain.

Everyday sound levels will seldom surpass 100 dB SPL. However, users of high power acoustic hearing aids can be exposed to much higher input pressures, with some modern hearing aids delivering sounds pressure levels as high as 140 dB (such as with the Phonak Naida Q-UP, Phonak, Switzerland). The middle ear then has to transport these very high sound intensities to the inner ear. In such circumstances, the contribution of middle ear nonlinearity may become important. In work on quasi-static pressure response, a strong nonlinear behaviour of both eardrum and stapes displacement has been reported (Dirckx and Decraemer 2001), which suggests that nonlinearities may also become important at high pressure acoustic inputs.

The cochlea itself also has strong nonlinear characteristics, resulting in the production of oto-acoustic emissions. These nonlinearities are generated in response to incoming sound which has passed through the middle ear. Therefore better knowledge of nonlinear characteristics of the incoming vibrations opens up the possibility to correctly measure otoacoustic emissions at higher sound pressure levels.

It is known that the ossicular chain shows some flexibility (e.g.Willi, Ferrazzini, and Huber, 2002, Funnell et al, 2005), and the 3D vibrations of the ossicles become complex at high frequencies (e.g. Hato, Stenfelt, & Goode, 2003). These observations show that the system is not just a simple lever. The individual ossicles themselves may also show some flexibility and change the vibration signal; modelling of the manubrium in cats, for example, has shown it to bend (Funnell, Khanna, and Decraemer, 1992). To quantify the nonlinear vibration input to the cochlea, measurements are therefore not only needed at the level of the umbo but also at the level of the footplate. Such measurements will show if additional nonlinearities are generated as sound is passed through the middle ear.

This paper reports measurements of the nonlinearities at the umbo and the footplate in rabbit ears. A very sensitive measurement technique, using multisine excitation signals specially designed for the detection of nonlinearities, was used to quantify the level of nonlinear vibration response at both the umbo and footplate.

2. Materials and methods

2.1 Measurement setup

Measurements were made on five ears removed from four adult male rabbits. The temporal bones were dissected from the skull and a hole was drilled in the bulla to expose the eardrum and allow free visual access perpendicular to the umbo. Next, the cochlea was

drilled away to expose the stapes footplate, and allow visual access perpendicular to that. Care was taken during this procedure to ensure that the annular ligament and the bone surrounding the footplate remained intact. The rabbit was chosen over the gerbil as an animal model because the complex preparation procedures have more chance of success in a larger ear.

With the middle ear exposed, small pieces (approximately 1 x 1 mm) of reflective tape (Polytec, Germany) were attached to the measurements points. One end of a small plastic tube was glued to the opening of the ear canal, while the other end was attached to an earphone speaker. The tube had a small hole drilled in its side through which the sound pressure level could be measured with a probe microphone (Bruel & Kjær, type 4182). Throughout the preparation, and between subsequent measurements, the specimen was kept moist using the vapour produced by an ultrasonic humidifier (Bionaire BT-204).

The vibrations of the umbo and footplate were measured with a laser vibrometer (Polytec model OFV-534) coupled to a surgical microscope. In this setup the laser spot was positioned using a motor controlled tilting mirror placed in front of the vibrometer. The signals to the speaker were designed in custom built software using Matlab. An A/D–D/A conversion board (RME HDSP 9632) with 24-bit resolution and a sampling rate of up to 192 kHz was used to generate and record the input and output signals.

Figure 1A shows a photograph of the rabbit ear as seen through the surgical microscope after preparation. This photograph is of a dried out specimen, taken some time after the measurements. The measurement points are indicated and the small pieces of reflective tape are visible. The direction of the laser beam is perpendicular to the plane of the photograph.

Figure 1B shows a snapshot of a 3D image of the rabbit ear based on micro-CT data. The sample was adjusted to try and ensure the laser was measuring at right angles to the footplate and at right angles to the tympanic ring.

All experiments were conducted in accordance with all relevant legislation and the directives set by our local ethics committee.

2.2 The multisine excitation method

The nonlinear distortions are measured using random phase multisines, which are described mathematically by the following equation:

$$S(t) = \frac{1}{\sqrt{N}} \sum_{k=1}^{N} A_k \sin(2\pi k f_{res} t + \varphi_k)$$

The signal is a multisine consisting of *N* harmonically related sines (all frequencies being multiples of f_{res}) with user defined amplitudes (A_k) and random phases (φ_k). The phases are randomly chosen such that the expected value $\varepsilon\{e^{i\phi k}\}$ is zero, for example, uniformly distributed in [0, 2π]. The excited harmonics are chosen by the user to be within a certain frequency band from f_{min} to f_{max}. The frequencies are spaced quasi-logarithmically, i.e. the harmonics are logarithmically spaced but chosen to coincide with the frequency grid determined by the frequency resolution f_{res}.

If a random phase multisine is used as the input to a system, a multisine will be recorded at the output. Each harmonic in the output signal will consist of a linear contribution from that harmonic in the input signal, and a nonlinear component from all the other harmonics. If a random phase multisine containing only odd harmonics is used at the input then only the odd degree nonlinearities in the system will contribute to the odd harmonics, the even degree nonlinearities will only contribute to the even, non-excited, harmonics.

Thus using only odd random phase multisines allows us to detect the level of even degree nonlinear distortions by measuring the even harmonics in the output spectrum. In order to determine the level of the odd degree distortions we need to randomly eliminate some odd harmonics from our input signal, and measure these at the output.

The measurement method proceeds as follows:

1. We first choose the measurements time (and thus the frequency resolution f_{res}), the frequency range (f_{min} and f_{max}), the frequency grid (linear or logarithmic) and the amplitude spectrum.

2. We divide up our odd excited harmonics into groups and randomly eliminate one harmonic from each group.

3. We randomly choose our phases (φ_k) and calculate our excitation signal, s(t). We output the signal and adjust its amplitude spectrum until we measure the desired stimulus levels.

4. We apply the signal to our system and measure P periods of the input ($s_i^P(t)$) and output ($s_o^P(t)$) signal.

From the P noisy output spectra $S_o^P(f)$, one can calculate the mean $\hat{S}_o$ at the excited harmonics f_{ex}, which gives the output response level, and the standard deviation of the mean $\sigma_{\hat{S}_o}$, which gives the noise level.

$$\hat{S}_o(f_{ex}) = \frac{1}{P}\sum_{p=1}^{P} S_o^P(f_{ex})$$

$$\sigma_{\hat{S}_o} = \sqrt{\frac{1}{P(P-1)}\sum_{p=1}^{P} |S_o^P(f_{ex}) - \hat{S}_o(f_{ex})|^2}$$

To get the level of the nonlinear distortions the output spectra $S_o^P(f)$ have to first be corrected for nonlinear distortions in the input spectra $S_i^P(f)$. Therefore the frequency response function has to be calculated at the excited harmonics f_{ex} for each measured period.

$$G^P(f_{ex}) = \frac{S_o^P(f_{ex})}{S_i^P(f_{ex})}$$

with p = 1,…, P.

If the test object behaves dominantly linear, a first order correction is obtained by subtracting the linear contribution of the test object from the output at the non-excited harmonics f_{nex}.

$$S_{oC}^P(f) = \begin{cases} S_o^P(f_{ex}) \\ S_o^P(f_{nex}) - G^P(f_{nex}) S_i^P(f_{nex}) \end{cases}$$

The frequency response at the non-excited harmonics is obtained by linear interpolation of the frequency response at the excited harmonics. Using these corrected output spectra, the level of the nonlinear distortions at the excited harmonics f_{ex} can be calculated by linear interpolation of the signal at the odd non-excited harmonics.

The signal used in the present measurements consisted of quasi logarithmically spaced frequencies between 125 and 16000 Hz. As explained before, the exited frequencies are harmonically related so they do not coincide exactly with logarithmically increasing frequency steps. Instead, harmonically related frequencies are used which are closest to the eight logarithmically spaced frequencies in each octave.

The number of excited harmonics should be large enough so that the middle ear dynamics are measured with a sufficiently large frequency resolution. Some prior knowledge or physical insight is typically used to set this number. In this case the prior knowledge was based on response measurements on a very fine frequency scale, showing smooth response curves which can be well represented by eight frequencies per octave. On the other hand the number of excited harmonics should not be too large in order not to deteriorate the signal-to-noise ratio per excited harmonic. Hence, a trade-off between

frequency resolution and signal-to-noise ratio must be made. When using more harmonics, the energy of the nonlinear response will be divided over more frequency lines, so detectability of nonlinearities will decrease. If we use less stimulation frequencies, the total level of the nonlinearities will remain the same, hence in each detection line the signal to noise ratio will be better so the appearance of nonlinearities will be detectable at a lower sound pressure level.

In the current study we chose to use eight frequencies per octave as a trade-off between good frequency resolution and good detectability. To optimise this choice a two-step procedure was followed: in a first measurement the signal-to-noise ratio was determined together with the dynamics, this information is then used to determine a good value of the number of excited harmonics in the second experiment. In our measurements we only excite the odd harmonics, the even harmonics are left unexcited for two reasons: (i) in order not to increase the variability of the estimated dynamics, and (ii) to distinguish the even from the odd nonlinear distortions at the output. The odd detection lines are selected by eliminating one randomly selected odd harmonic out of two or three consecutive harmonics. Proceeding in this way the level of the odd nonlinear distortion at the excited odd harmonics is given by the level of the nearest non-excited odd harmonic (Schoukens et al. 2009).

Using this method we measured the vibration response, nonlinear distortions, and noise level in five rabbit ears at eleven sound pressure levels ranging from 90 dB SPL to 120 dB SPL in steps of 3 dB.

3. Results

The data we recorded is three dimensional: vibration responses and distortion levels are a function of both frequency and sound pressure level. So, we will present two sets of graphs, one set showing data as a function of frequency at different sound pressure levels, the other set showing data as a function of sound pressure level at different frequency octave bands. To give an idea of inter specimen variability we will first show data for all ears at one sound pressure level and in one frequency band. Then we will show averaged data in more detail.

At 120dB one of the ears showed anomalous behavior, we suppose some technical problem generated this deviation, but we could not pinpoint its source. So, for all further calculations we used all five ears except for the sound pressure level of 120 dB where we only used 4 ears and left out the outlier. In presenting the results from individual ears, we therefore used data measured at 117 dB, so as to show five ears.

Effect of removing the cochlea

In order to be able to measure on the footplate, the cochlea needed to be removed. To examine the effect of this procedure we measured the vibration response and nonlinear distortions at the Umbo before and after drilling open the cochlea. We measured on three separate temporal bones; the results are shown in figure 2.

The vibration response of the three bones barely showed any change after removing the cochlea. Only at the resonance peak at around 1800 Hz is a difference noticeable, with the response generally showing less of a peak with the cochlea intact. The nonlinear distortions show slightly more variation, and the nonlinearities appear over most frequencies to be slightly lower than the level with the cochlea intact.

Vibration response and nonlinearities in Individual ears

Figure 3A shows the vibration response measured at the umbo for an input sound pressure level of 117 dB. Figure 3B shows the data obtained at the stapes. We see that there is little variability among the different ears, and that the stapes curves resemble the curves obtained at the umbo. Figure 3C shows the ratio of umbo vibration response to stapes vibration response for all five ears: the ratio is nearly constant at about 10dB over the entire frequency range between 125 Hz and 4 kHz, but slightly diminishes at the higher frequencies.

Figure 4A shows the level of the nonlinearities measured at the umbo for a sound pressure level of 117 dB. There is more variability between ears than for the vibration response, but the curves are still close to each other. Comparing these curves to the vibration response presented in Figure 3A, we see that the nonlinearities are about 40 dB lower. Comparable results were obtained at all other sound pressure levels. In Figure 4B we see the nonlinearities measured at the stapes: again variability between ears is a bit larger than for the vibration response, but it is still rather limited. Figure 4C then shows the ratio of nonlinearity levels at the umbo and at stapes. All curves are again rather close, but there is of course some more variability between measurement points. On average the ratio is again on the order of 10 dB, just like the ratio of the vibration responses, except at higher frequencies where there appears a slight tendency for the ratio to decrease.

Average results

For all following figures, an average was taken over the five measured ears, except at a sound pressure level of 120 dB where only four ears were used. In all plots we have added whiskers to indicate the standard deviation. All calculations were done using dB values. While this is not the same as performing the calculations using linear values, this is commonly done when reporting audiological data and serves to give an impression of the variability in the data.

Figure 5 shows the total vibration response (squares) and nonlinear distortions (triangles) as a function of frequency for sound pressure levels of 90 dB (A), 108 dB (B) and 117 dB (C) for the umbo (red) and the stapes (blue). The noise floor is indicated by the dashed line. For frequencies between 125 Hz and 1000 Hz the vibration response curves are rather flat, at 1800 - 1900 Hz we see a small resonance peak, and at higher frequencies the response curve becomes a bit more complicated and shows a gradual roll-off. The shape of the response curves of the stapes and umbo are largely identical, and remain practically the same for increasing sound pressure levels. At 90 dB SPL the nonlinearities hardly exceed the noise, but at 120 dB SPL they are clearly visible. The nonlinearity curves obtained for umbo and stapes have a rather good resemblance. Standard deviations on the data points are moderately larger for the nonlinearities than for the vibration response as the level is much lower and the contribution of noise becomes more important.

In order to better evaluate differences between umbo and stapes response, figure 6 shows the ratio of umbo to stapes vibration response (red) and nonlinearities (blue) as a function of frequency, measured at 117 dB SPL. The vibration response ratio is fairly flat across the entire frequency range. For frequencies ranging from 125 Hz to about 4 kHz the ratio is between 10.5 dB and 12 dB. At lower sound pressure levels we found similar results, with a slight increase of the ratio to about 13 -16 dB at 90dB SPL.

The ratio of the nonlinear distortions shows more variability than the ratio of vibration response. The standard deviations are larger than for the vibration response curve, but for frequencies between 250 Hz and 8 kHz we find a ratio of about 5 to 15 dB. At the lowest measured frequency (125 Hz) the ratio drops to just under 0 dB, but it should be noticed that at such low frequencies the sensitivity of the vibrometer goes down and noise level increases.

Figure 7 shows the total vibration response (squares) and nonlinear distortions (triangles) as a function of sound pressure level for the frequency bands 250-500 Hz (A), 1-2 kHz (B) and 4-8 kHz (C), for the umbo (red) and the stapes (blue). Error bars again indicate the standard deviation over the individual ears, and the noise floor is indicated by the dashed line. In all figures we see similar behaviours: the response curves of umbo and stapes are practically

parallel, the distance between the nonlinearity curves varies a bit more but is also rather constant. In the 1-2 kHz octave the nonlinearity of the umbo response begins to rise above the noise level at 93 dB, and for the stapes it rises above the noise at 99 dB. In the 4-8 kHz octave the Stapes response rises above the noise at 102 dB SPL. In the lowest octave, 250 - 500 Hz, the noise floor is somewhat higher, and the nonlinearity in the stapes response becomes visible at 108 dB SPL.

In Figure 8 we show the ratio of umbo vibration response to stapes vibration response (red), and the ratio of the nonlinearity of umbo vibration response to stapes vibration response (blue), for the frequency bands 250-500 Hz (A), 1-2 kHz (B) and 4-8 kHz (C). For the nonlinearities, data is only shown when the signals for both umbo and stapes are both above the noise floor. In all three octaves we see that the ratio of umbo vibration response to stapes vibration response is a nearly flat function of sound pressure level. In the 250-500 Hz octave the ratio is 7 dB, while in the 1-2 kHz and 4-8 kHz octaves it has a slightly higher value of around 9 dB. At the very highest sound pressure level of 120 dB SPL we see a small drop in the ratio of about 2 dB in all octaves. The ratio of umbo nonlinearity to stapes nonlinearity is somewhat less constant as a function of sound pressure level, but variations are small. In all octaves we see that the nonlinearity ratio has a slight tendency to increase as a function of sound pressure level, meaning that nonlinearities at the level of the umbo rise slightly faster, as a function of sound pressure level, than nonlinearities at the level of the stapes, but the effect is limited to less than 7 dB in all octaves.

In Figure 9 we finally try to give a three dimensional representation of our results. The figure shows the ratio of the level of nonlinearity to the level of vibration response as a function of both frequency and displacement level for the umbo (top graph) and the stapes (bottom graph). Colour is used to represent the ratio, and the vibration response curves for sound pressure levels of 108 dB, 114 dB and 120 dB have been added as solid black lines. The ratio of nonlinearity to vibration response is only shown in zones where the level of nonlinearity exceeds the noise level. When comparing both graphs we see that they closely resemble each other, showing that the nonlinear behaviour at the level of the stapes is to some extent the same as at the level of the umbo. At 120 dB SPL the vibration amplitude of the umbo is around -15 dB (re 1 μm) , and the nonlinearity levels are about 40 dB below the response levels for frequencies between 125-1000 Hz. In the resonance peak at 1800 - 1900 Hz, the vibration response increases to -2 dB (re 1 μm). As a reference, we have added a red dotted line of -15dB vibration amplitude in the graph. Along this line of constant vibration level of -15 dB (re 1 μm) we see that the level of nonlinearities drops to -70 dB below the vibration response.

4. Discussion

Animal Model

The availability of fresh human material is very limited, which induces the need to switch to animal models in the development of a new measurement technique. The rabbit ear differs significantly from the human ear, but they do share several features. The hearing range of the common rabbit was determined by Heffner using behavioral audiometry (Heffner, 1980). His experiments showed that at a sound pressure level of 60dB SPL the lower frequency boundary of hearing in rabbit is 96Hz, and the upper boundary is 49kHz. This frequency range partly overlaps with the human auditory range. The rabbit eardrum itself is thinner than in humans (Buytaert et al. 2013), but the visco-elastic properties of the rabbit ear (Aernouts et al. 2010), are in the same range as those measured in humans (De Greef et al. 2014). Both human and rabbit ears do, of course, have three-ossicles with ligaments and tendons.

For interpretation of the current results, the most significant difference might be that in humans the incudo-malleolar joint has been shown to be mobile at moderate sound pressure levels (Willi et al. 2002) while in rabbits both ossicles are fused together (Soons et al. 2010), allowing hardly any relative motion. So, in humans the motion in the articulation between the incus and malleus may have an additional influence on the nonlinearities at the level of the stapes.

Influence of the Cochlea

In our procedure we measured the motion of the footplate after removing the cochlea. This approach allows us to measure perpendicular to the footplate, and also allows us to purely test the middle ear system. The cochlea is known to possess highly nonlinear characteristics due to its active feedback mechanisms, and it has been shown that the cochlea is able to generate nonlinear distortions in the middle ear post mortem (Rhode 2007). Thus, removing it eliminates the potential for these distortions, and we can be sure that what we measured was entirely due to the middle ear system and not due to any remaining cochlear activity. A further reason for choosing to remove the cochlea was due to the relative inaccessibility of the stapes footplate in rabbits. In a preparatory experiment we measured vibration response at the umbo before and after removal of the cochlea and we found only limited changes in both vibration response and the level of the nonlinearities (Figure 2). The vibration response remained practically unchanged for frequencies below 1 kHz but at higher frequencies some

changes were observed and vibration amplitudes slightly increased. The level of the nonlinearities also remained largely unchanged, except at some of the lower frequencies where some increase is seen. Overall the level of the nonlinearities did not change strongly, but some effect of removal of the cochlea proved to be inevitable.

Vibration Response

At low frequencies the response curve is rather flat, gradually increasing to a resonance peak, and for frequencies beyond this first resonance the curves become more complicated. The vibration response at level of the stapes is largely a near-perfect copy of the vibration response of the umbo, only 10 dB lower. In Figure 5 we see that the ratio is very constant up to frequencies of 4 kHz, but at the highest frequencies the ratio of both responses somewhat drops from 10 dB at 4 kHz to 0 dB at 16 kHz. Here we should emphasize that we were only able to measure along one direction, and did not measure the full three dimensional motion of the ossicles. It has been shown that at low frequencies the stapes performs a piston-like motion (Huber et al. 2001; Hato et al. 2003) but that at high frequencies the motions become much more complicated. It is therefore possible that in the 8-16 kHz octave our single-direction measurement underestimates the maximal amplitude of stapes motion.

In Figure 8 we have shown the ratio of umbo vibration response to stapes vibration response as a function of input sound pressure level. We have already described how the ratio of umbo to stapes vibration response is practically constant, and we now see that the ratio of nonlinear response also shows very little dependence on sound pressure level. At the lower pressure levels the ratio is a little bit less than the ratio of the vibration response, and there is a slight tendency for the ratio to increase at higher pressure levels. This means that at the at the highest sound pressure levels the vibrations at the footplate show a bit less nonlinearity than the vibrations at the umbo. If the sound transmission from malleus to stapes would not have any influence on the non-linearities, we would expect the ratio to be equal to the ratio of the vibration amplitudes. Apparently transmission of the sound along the ossicular chain has some effect on the level of non-linearities, but the effect is marginal. At this instance we do not have an explanation for the fact that at higher sound pressure levels the stapes vibration response is a bit less non-linear than umbo vibrations response. In any case the results show that the joints between the ossicles do not increase the non-linearities in the vibration signal going to the cochlea.

Nonlinearities as a function of frequency

From Figure 5C we see that the curves showing the level of nonlinearities for umbo and stapes resemble the curves of vibration response of both ossicles, but that there are also important differences. Before the first resonance peak the curves are rather flat, just like the vibration response curves. However, at the location of the first resonance (1800 - 1900 Hz) the nonlinearity curves stay flat and do not show the same characteristic maximum. Beyond the first resonance, the level of the nonlinearities shows a stronger decrease as a function of frequency than the vibration response. We will come back to this observation when discussing the 3D colour representation of the data.

In Figure 6 we have shown the ratio of the nonlinearities between the umbo and the stapes. The gap between the nonlinearity curves is rather constant over the entire frequency range, although the variability between data points is much larger than for the vibration response. The ratio of nonlinearities remains largely between 5 and 15 dB. Here we should remember that the levels of the nonlinearities are 40 dB to 70 dB lower than the levels of the vibration amplitudes (see Figure 5), making the data much more noise sensitive. On the whole however we see that the ratio between umbo and stapes vibration response and the ratio between umbo and stapes nonlinearity as function of frequency show good resemblance. We conclude that no significant nonlinearities are added to the vibration response when vibrations are passed on from the umbo to the stapes.

Nonlinearities as a function of sound pressure level

In Figure 7 we showed how the vibration amplitudes and the level of nonlinearities increase as a function of input sound pressure level. With our detection method, nonlinearities become detectable at vibration amplitudes as low as -110 dB (re 1 µm) in the 1-2 kHz and 4-8 kHz octaves. In the 250-500 Hz octave detectability is a bit less (about -90dB re 1 µm). The reason is that laser vibrometry is a velocity sensitive technique, so measuring sensitivity increases as a function of frequency. We have also seen that nonlinearities at the umbo can be detected at sound pressure levels of 6 dB to 10 dB lower than for the stapes. It is important to realise that these findings do not mean that nonlinearities are absent at lower sound pressure levels, it only means that our method is not able to detect them at this point. So, the nonlinearity detected at the umbo at 93 dB is probably also reflected at the level of the stapes, but the nonlinear behaviour of the stapes remains below the detection level of the current method. Since the vibration level at the umbo is about 10 dB higher than at the stapes, it seems logical that more sound pressure is needed for stapes nonlinearity to

become detectable. This observation also emphasises the fact the level at which the nonlinearities become detectable is of little interest in itself.

More important therefore is the way the nonlinearity levels change as function of input sound pressure. When we compare the nonlinearity curves to the vibration response curves, we clearly see that the level of nonlinearities increases more strongly as a function of sound pressure level than the vibration response. When we make a linear fit to the output response data as function of input sound pressure level, we find the gradient of the line to be practically equal to one across all frequency bands. However, when we make a linear fit for the nonlinearity data, we find the gradient to range from 1.7 to 2 in different frequency bands.

In Figure 8 we have shown the ratio of umbo nonlinearity to stapes nonlinearity as a function of sound pressure level. We have already described how the ratio of umbo to stapes vibration response is practically constant, and we now see that the ratio of nonlinear response also shows very little dependence on sound pressure level. There is apparent a slight tendency for the ratio to increase at higher pressure levels, meaning that at the highest sound pressure levels the vibrations at the footplate show a bit less nonlinearity than the vibrations at the umbo. At this instance we do not have an explanation for this observation, and the effect is rather marginal. But some increasing “linearisation” of the vibration signal is observed.

Nonlinearity as function of frequency at constant vibration level

We already mentioned above that the curves of nonlinearity as a function of frequency do not show a peak in the frequency range corresponding to the resonance peak observed in the vibration response curve. This observation is emphasized when looking at lines of constant vibration amplitude in Figure 9. When we look at a vibration amplitude of -15 dB (re µm) in the plot for the umbo, we see that this vibration response is reached at 120 dB SPL for a frequency range between 125 Hz and 500 Hz. At frequencies going up to the resonance peak the same vibration level is reached at lower sound pressure levels. When looking at the nonlinearities however we see that in the region 125-500 Hz the level of umbo nonlinear response is about 40 dB below vibration response, while at the resonance peak the nonlinearities are more than 70 dB below vibration response. We find this a most peculiar observation: apparently it is not (only) the amplitude of the motion which determines the magnitude of the nonlinearity, as the same motion amplitude at one frequency results on 30 dB more nonlinearity as the same vibration amplitude at another frequency. In general we

see that, for the same vibration level, the nonlinearities are much more prominent at low frequencies than at high frequencies.

Source of the nonlinearities.

Pinpointing the sources of the nonlinear responses we measured is beyond the scope of the current paper, and will need a detailed modelling approach which we are planning for future work. Possible sources of the nonlinear response are the nonlinear elastic properties and damping properties of the eardrum and the ligaments, and the asymmetrical shape of the eardrum. In quasi-static studies it has been shown that umbo displacement as a function of pressure shows hysteresis (Dirckx et al. 2006) and is also asymmetric, which obviously causes a nonlinear response, and it has been suggested that the tent-like shape of the eardrum may play an important role in this observation. For the quasi-static pressure range at large amplitudes (2 kPa), modelling has shown that the asymmetrical shape of the eardrum is a crucial factor (Ladak et al. 2006). In the acoustic range displacements are far smaller, but still the asymmetrical shape of the membrane may influence manubrium motion. It is our current hypothesis that membrane geometry asymmetry is the major cause of middle ear nonlinear response, and we will investigate this further both through modelling approach and measurements in species with flat eardrums. From the current measurements we see that the nonlinearities observed at the stapes are largely identical to the nonlinearities seen at the umbo. This leads us to believe that the eardrum is the governing component in the nonlinear response of the middle ear, and it certainly shows that no significant additional nonlinearities are added to the vibration signal on its way through the middle ear.

5. Summary and Conclusion

In this paper we measured the vibration response and the nonlinearities in the vibration response of the umbo and the stapes. Using a very sensitive measurement technique we were able to detect nonlinearities at input sound pressure levels of 93 dB SPL. In general, we found that nonlinearities as function of frequency and as function of sound pressure largely show the same behaviour for both the umbo and the stapes. Further research will be needed to pinpoint the source of these nonlinearities, but the current results show that no significant additional nonlinearity is generated as the vibration signal is passed through the middle ear chain. As we do not see major differences between the nonlinear response

measured at the stapes and that measured at the umbo, the current results suggest that measurements at the level of the eardrum are sufficient to detect middle ear nonlinearity.

Nonlinearities are most prominent in the lower frequencies (125 Hz to 1 kHz), where their level is about 40 dB below vibration response at an input of 120 dB SPL. At lower sound pressure levels nonlinearities are less prominent but we have demonstrated their presence, and correct quantification of the effect may be relevant in the interpretation of nonlinear cochlear responses measured through oto-acoustic emissions at higher sound pressure levels. We also found that nonlinearities increase nearly twice as fast as the vibration response when sound pressure levels increased. This indicates that middle ear nonlinear distortions may become important when developing high power hearing aids which generate sound pressure levels as high as 140 dB.

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Figure Captions

Fig. 1 (A) photograph of the prepared specimen with the measurement points indicated, (B) a 3-D rendering of the ossicular chain obtained from a CT scan. In (B) the direction of the laser beams is indicated by the red dashed line, in (A) the laser beam direction is perpendicular to the plane of the photograph. In figure 1B the tympanic membrane is coloured red, the malleus blue, the incus green, and the stapes purple.

Fig.2 Vibration response (squares) and amplitude of non-linearities (triangles) measured at the umbo before (blue) and after (red) removing the cochlea. This data was obtained at a sound pressure level of 117 dB.

Fig. 3 Vibration amplitude measured in five individual ears as a function of frequency; (A): umbo, (B): stapes; (C) ratio of umbo to stapes vibration amplitude

Fig. 4 Amplitude of non linearities measured in five individual ears as a function of frequency; (A): umbo, (B): stapes; (C) ratio of umbo to stapes non-linearity amplitude

Fig. 5 Vibration response and non-linearities amplitude for umbo and stapes as a function of frequency. Data is averaged over five ears, and standard deviations are added; measurements made at (A) 90 dB SPL, (B) 108 dB SPL, (C) 120 dB SPL.

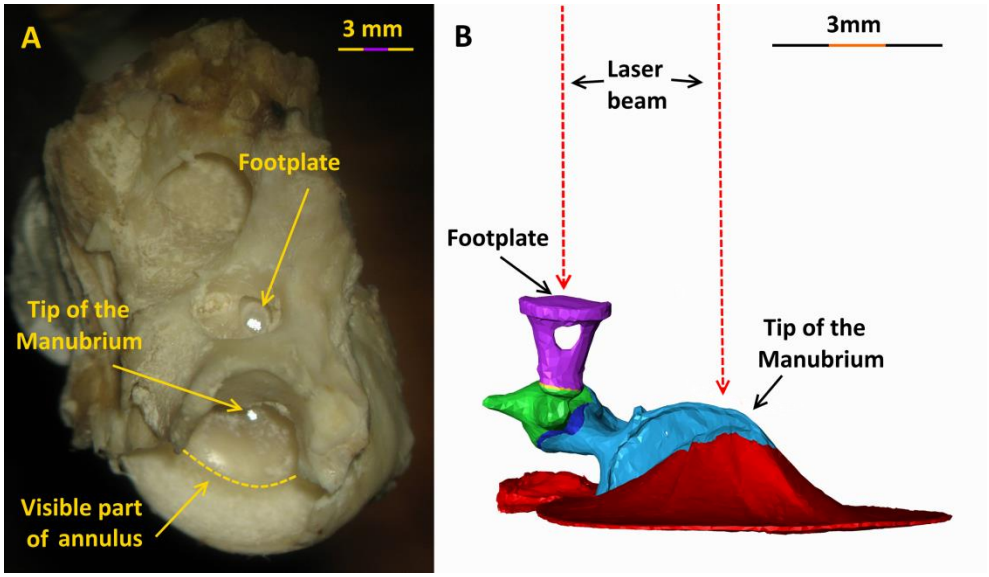
Fig. 6 Ratio of umbo to stapes vibration amplitude (diamonds) and nonlinearity amplitude (squares) as a function of frequency at 117dB SPL sound pressure level. Data is averaged over five ears, and standard deviations are added.

Fig. 7 Vibration response and non-linearities amplitude for umbo and stapes as a function of sound pressure level. Data is averaged over five ears, and standard deviations are added; data obtained in frequency band (A) 125Hz to 500 Hz, (B) 1kHz to 2kHz, (C) 4kHz to 8kHz.

Fig. 8 Ratio of umbo to stapes vibration amplitude (diamonds) and nonlinearity amplitude (squares) as a function of sound pressure level. Data is averaged over five ears, and standard deviations are added. Data obtained in the frequency bands (A) 125Hz to 500 Hz, (B) 1kHz to 2kHz, (C) 4kHz to 8kHz.

Fig. 9 Vibration amplitude and ratio of non-linear distortion to vibration response as a function of frequency. Top graph: umbo; bottom graph: stapes. Dotted lines indicate vibration responses at 180 dB, 114 dB and 120 dB sound pressure level. Ratio of distortions to vibration response is indicated in colour. The horizontal line is added as a reference to see how the ratio changes as function of frequency for fixed level of vibration amplitude.

549 **Figure 1**



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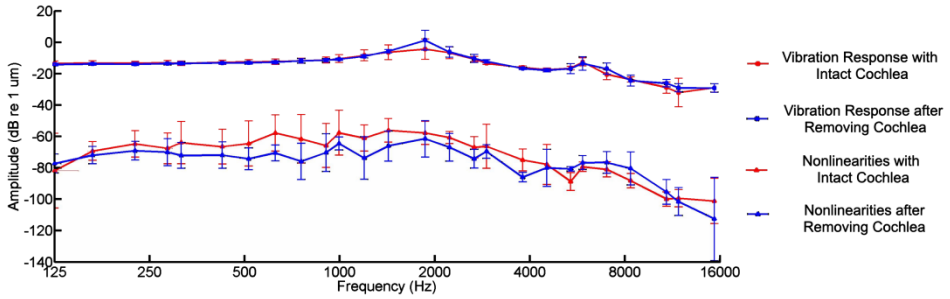
551 **Figure 2**



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553 **Figure 3**

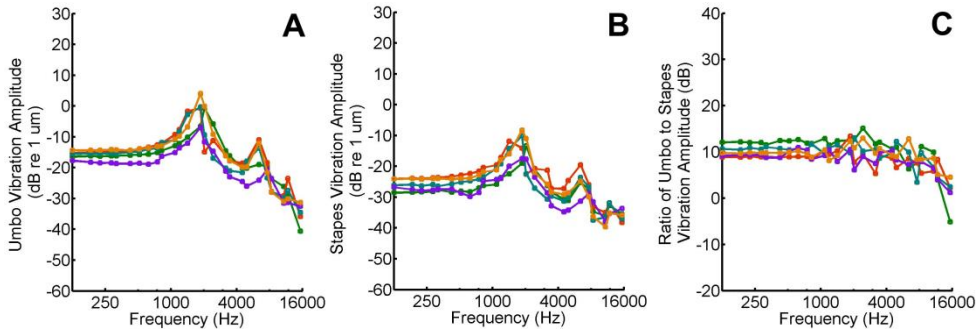

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Figure 4

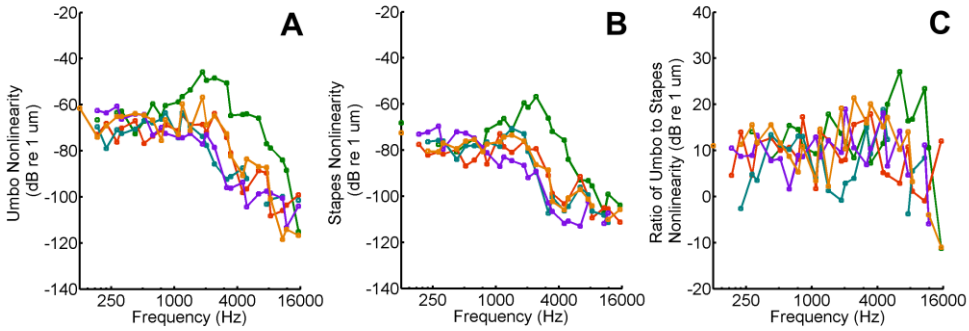
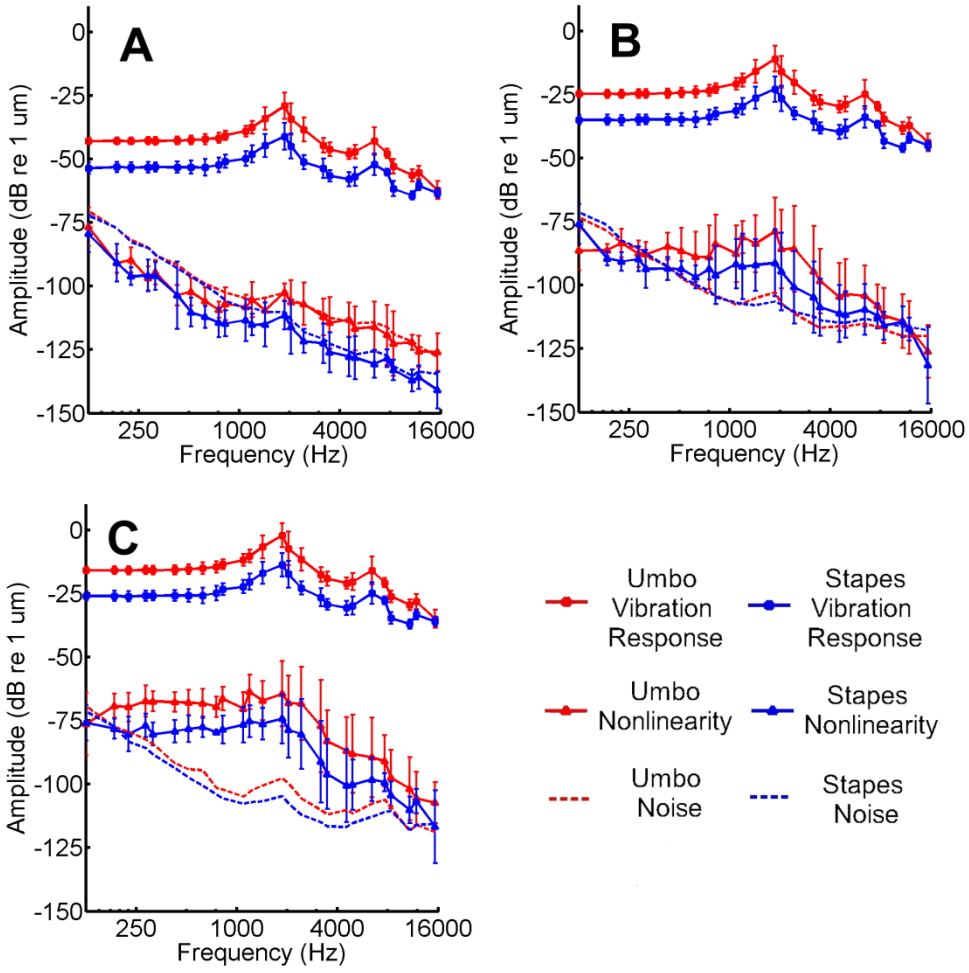


Figure 5



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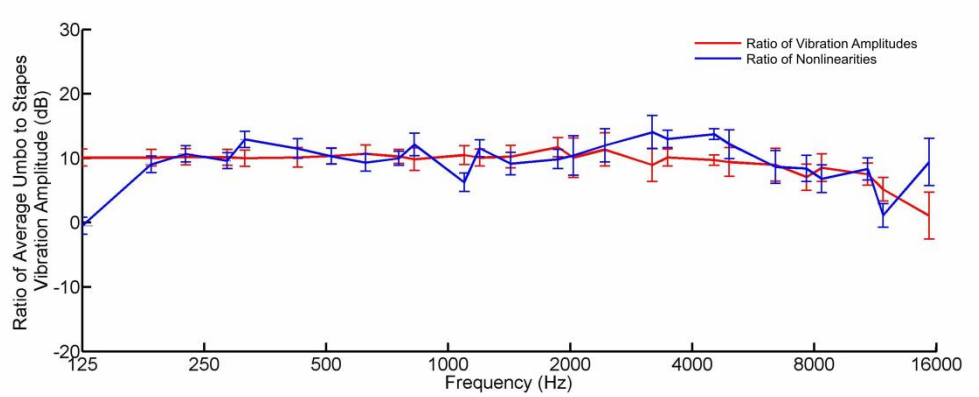

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563 **Figure 7**

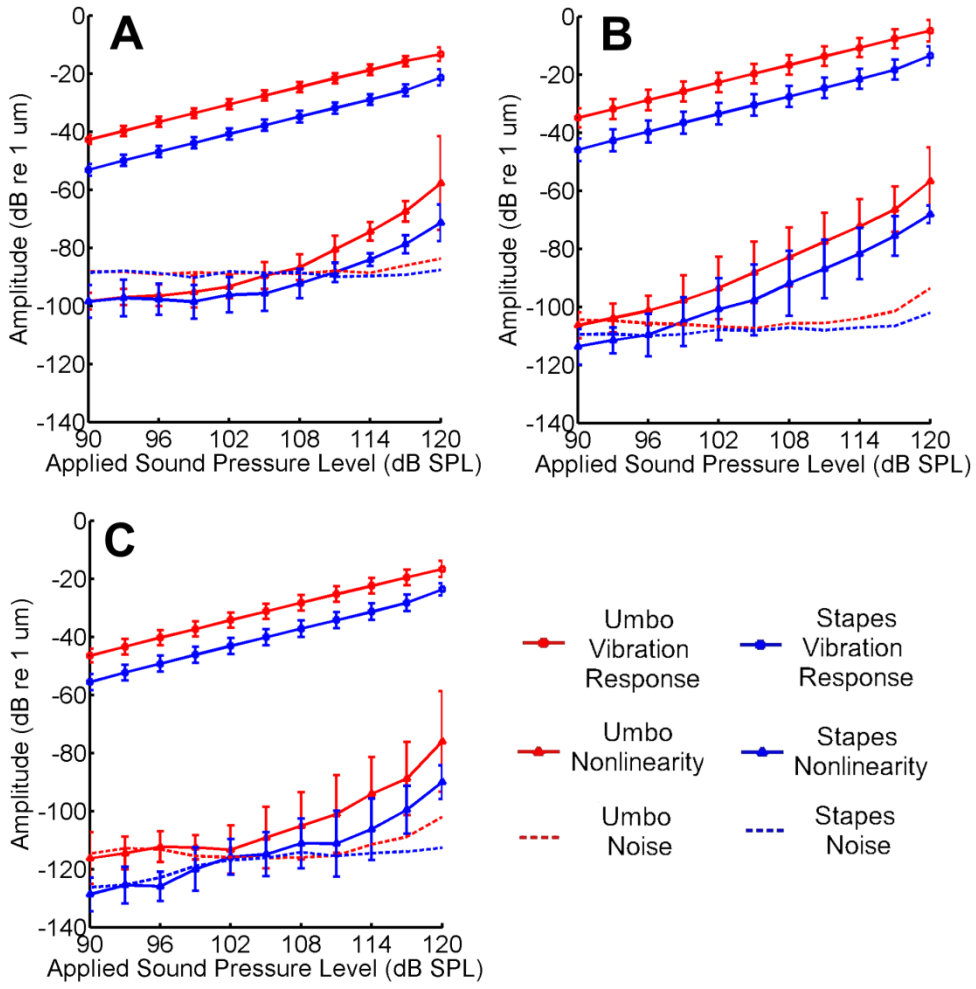

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Figure 8

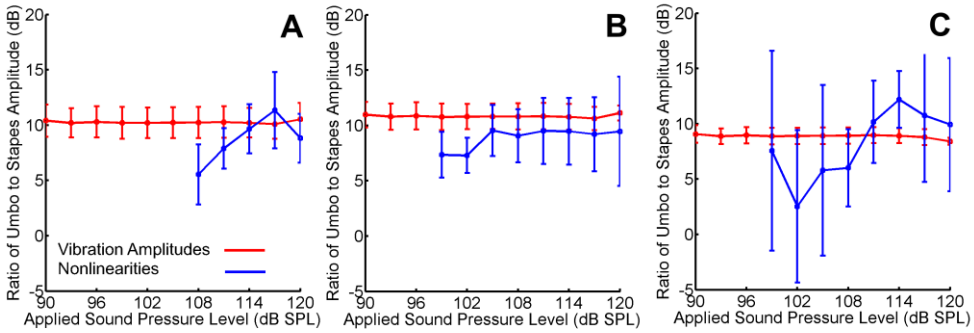

Figure 9