Research Article

Nonlinear Vibration Response Measured at Umbo and Stapes in the Rabbit Middle ear

JOHN PEACOCK,¹ RIK PINTELON,² AND JORIS DIRCKX¹

¹Laboratory of Biomedical Physics, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

²Department of Fundamental Electricity and Instrumentation (ELEC), Vrije Universiteit Brussel, Plenlaan 2, 1050 Brussels, Belgium

Received: 17 January 2014; Accepted: 24 June 2015; Online publication: 11 July 2015

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. The current results show that no significant additional nonlinearity is generated as the vibration signal is passed 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. The level of nonlinearities rises with a factor of nearly 2 as a function of sound pressure level, indicating that they may become important at very high sound pressure levels such as those used in high-power hearing aids.

Keywords: middle ear, nonlinear distortions, laser vibrometry, multisine

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 sound pressure level (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 SPLs 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 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.

Every day, 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 SPLs 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 the middle ear's quasi-static pressure response, a strong nonlinear behavior of both eardrum and stapes displacement has

Correspondence to: John Peacock · Laboratory of Biomedical Physics · University of Antwerp · Groenenborgerlaan 171, 2020 Antwerpen, Belgium. email: john.peacock@uantwerpen.be

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 otoacoustic emissions. These nonlinearities are generated in response to incoming sound which has passed through the middle ear. Therefore, better knowledge of the nonlinear characteristics of the incoming vibrations opens up the possibility to correctly measure otoacoustic emissions at higher SPLs.

It is known that the ossicular chain shows some flexibility (e.g., Willi et al. 2002; Funnell et al. 2005) and that the 3D vibrations of the ossicles become complex at high frequencies (e.g., Hato et al. 2003). These observations show that the system is not just a simple lever. The individual ossicles themselves may also show some flexibility and thus modify the vibration signal; modeling of the manubrium in cats, for example, has shown it to bend (Funnell et al. 1992). To quantify the nonlinear vibration input to the cochlea, measurements are therefore needed not only 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 the footplate.

MATERIALS AND METHODS

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 1×1 mm) of reflective tape (Polytec, Germany) were attached to the measurement 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 SPL could be measured with a probe microphone (Brüel

& Kjær, type 4182). Throughout the preparation, and between measurements, the specimen was kept moist using vapor 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.

A single laser vibrometer was used. With a custom-made pico-motor-controlled mirror setup, the laser beam could be moved around in the field of view. Between measurements, this mirror assembly was used to aim the laser beam at the reflective targets on the stapes or the umbo. A drawing of the microscope setup can be found in Peacock et al. (2014).

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 that 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 relevant legislation and the directives set by our local ethics committee.

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 $\mathcal{E}\{e^{j\varphi_k}\}$ is zero, for example, uniformly distributed in $[0, 2\pi]$. The excited har-

FIG. 1. **A** Photograph of the prepared specimen with the measurement points indicated and **B** a 3D 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 **B** the tympanic membrane is colored red, the malleus blue, the incus green, and the stapes purple.

monics are chosen by the user to be within a certain frequency band from f_{min} to f_{max} .

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 component from that harmonic in the input signal and a nonlinear component from all harmonics. If a random-phase multisine containing only odd harmonics is used at the input to a system, then only the odd-degree nonlinearities will contribute to the odd harmonics; the even-degree nonlinearities will only contribute to the even 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 (Schoukens et al. 2009).

The measurement method proceeds as follows:

1. We first choose the measurement 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 equal groups of consecutive harmonics (e.g., two, three, or four) 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 $S_o^P(f)$, we 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.

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

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

The level of the nonlinear distortions is found by looking at the nonexcited frequencies in the output spectra $S_o^P(f)$. However, distortions in the input signal will also contribute to the output and these must be corrected for. This is done by measuring the input signal at the nonexcited frequencies and subtracting this from the output after multiplication by the system's frequency response. To do this, we first have to calculate the frequency response function at the excited harmonics (f_{ex}) for each measured period.

$$G^P(f_{ex}) = \frac{S_o^P(f_{ex})}{S_i^P(f_{ex})} \text{ with } p = 1, \dots, P.$$

The frequency response function at the nonexcited harmonics $G^P(f_{nex})$ is obtained by linear interpolation of the frequency response at the excited harmonics $G^P(f_{ex})$. The true output spectra $S_{oc}^P(f)$ at the excited (f_{ex}) and nonexcited (f_{nex}) harmonics is then exactly recovered as

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

(Pintelon and Schoukens 2013). A correction is not necessary for the excited harmonics since any distortions present at these frequencies are just part of the excitation signal. Using the corrected output spectra, the level of the nonlinear distortions at the excited harmonics is calculated by linear interpolation of the absolute value of the signal at the odd nonexcited harmonics.

The signal used in the present measurements consisted of quasi-logarithmically spaced frequencies between 125 and 16,000 Hz, i.e., the harmonics are logarithmically spaced but chosen to coincide with the frequency grid determined by the frequency resolution f_{res} . In other words, frequencies are used which are closest to the eight logarithmically spaced frequencies in each octave.

The larger the number of harmonics used, the greater the frequency resolution; however, more harmonics leads to a lower signal-to-noise ratio per harmonic. When using more harmonics, the signal-to-noise ratio per harmonic decreases, while the signal-to-nonlinear-distortion ratio remains the same (Schoukens et al. 2009), so the detectability of nonlinearities will decrease. If we use less stimulation frequencies, the signal-to-noise ratio will be better, and thus, 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 optimize this choice, a two-step procedure was followed: In the 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 nonexcited odd harmonic (Schoukens et al. 2009).

Using this method, we measured the vibration response, nonlinear distortions, and noise level in five rabbit ears at 11 sound pressure levels ranging from 90 to 120-dB SPL in steps of 3 dB.

RESULTS

The data that we recorded are 3D: Vibration responses and distortion levels are a function of both

frequency and sound pressure level. Therefore, we will present two sets of graphs, one set showing data as a function of frequency at different sound pressure levels and the other set showing data as a function of sound pressure level at different frequency bands. To give an idea of interspecimen 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 120-dB SPL, one of the ears showed anomalous behavior; we suppose that 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 four 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 all five.

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 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 appear to be slightly lower than the level measured with the cochlea intact over most frequencies.

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 10 dB over the entire frequency range between 125 Hz and 4 kHz but slightly diminishes at the higher frequencies.

In Figure 4 we show data for the nonlinearities. In some measurements, and at some frequencies, the level of the nonlinearity was below the noise floor and is therefore not plotted.

FIG. 2. Vibration response (*squares*) and amplitude of nonlinearities (*triangles*) measured at the umbo before (*blue*) and after (*red*) removing the cochlea. These data were obtained at a sound pressure level of 117 dB.

Figure 4A shows the level of the nonlinearities measured at the umbo for a sound pressure level of 117 dB. These curves show much more variability between the ears than for the vibration response. One measurement showed the level of the nonlinear distortions to be much higher than in the others, yet all showed a similar pattern across frequencies, and for most measurements, the variation was within 10 dB. Comparing these curves to the vibration response presented in Figure 3A, we see that the nonlinearities are about 40 dB lower.

In Figure 4B we see the nonlinearities measured at the stapes. One measurement showed larger nonlinearities than the others at some frequencies, but most of the measurements were within 10 dB. Finally, in Figure 4C we see the ratio of umbo to stapes nonlinearity. On average, the ratio is 10 dB, the same as the ratio of the vibration responses. However, more variation between individual measurements is seen, particularly at the highest frequencies. Similar to the vibration response ratio in Figure 3C, the nonlinearity ratio also shows a slight tendency to decrease at higher frequencies.

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 decibel 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.

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 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 is largely identical and remains practically the same for increasing sound pressure levels. At 90-dB SPL, the nonlinearities hardly exceed the noise, but at 117-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

FIG. 3. Vibration amplitude measured in five individual ears as a function of frequency: **A** umbo and **B** stapes and **C** ratio of umbo to stapes vibration amplitude.

FIG. 4. Amplitude of nonlinearities measured in five individual ears as a function of frequency: **A** umbo and **B** stapes and **C** ratio of umbo to stapes nonlinearity amplitude.

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 to about 4 kHz, the ratio is between 10.5 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 90-dB 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 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 noted that at such

FIG. 5. Vibration response and nonlinearity amplitude for umbo and stapes as a function of frequency. Data are averaged over five ears, and standard deviations are added, measurements made at **A** 90-dB SPL, **B** 108-dB SPL, and **C** 117-dB SPL.

FIG. 6. Ratio of umbo to stapes vibration amplitude (*red*) and nonlinearity amplitude (*blue*) as a function of frequency at 117-dB SPL. Data are averaged over five ears, and standard deviations are added.

low frequencies, the sensitivity of the vibrometer goes down and the 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 indi-

vidual ears, and the noise floor is indicated by the dashed line. Each octave contains three or four different excited harmonics, these values are calculated by taking the average of each of the frequencies for each ear and then calculating the mean and standard deviation across all ears. In all figures, we see similar behavior: The response curves of umbo and stapes are practically parallel, while the distance between the

FIG. 7. Vibration response and nonlinearity amplitude for umbo and stapes as a function of sound pressure level. Data are averaged over five ears, and standard deviations are added, data obtained in frequency band **A** 125 to 500 Hz, **B** 1 to 2 kHz, and **C** 4 to 8 kHz.

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 SPL, 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 are only shown when the signal for both umbo and stapes is 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 3D 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). Color is used to represent the ratio, and the vibration response curves for sound pressure levels of 108, 114, and 120 dB have been added as dotted 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 behavior 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 $1 \mu\text{m}$), and the nonlinearity levels are about 40 dB below the response levels for frequencies between 125 and 1000 Hz. In the resonance peak at 1800–1900 Hz, the vibration response increases to -2 dB (re $1 \mu\text{m}$). As a reference, we have added a red dotted line at -15 dB (re $1 \mu\text{m}$) to the graph. Along this line of constant vibration level, we see that the level of nonlinearities drops to -70 dB below the vibration response.

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 60-dB SPL, the lower frequency boundary of hearing in rabbits is 96 Hz, and the upper boundary is 49 kHz. This frequency range partly overlaps with the human auditory range. The rabbit eardrum itself is thinner than the human eardrum (Buytaert et al. 2013), but its viscoelastic properties (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

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

FIG. 9. Vibration amplitude and ratio of nonlinear distortion to vibration response as a function of frequency. *Top graph:* umbo; *bottom graph:* stapes. Dotted lines indicate vibration responses at 108, 114, and 120-dB sound pressure level. Ratio of distortions to

vibration response is indicated in color. The *horizontal line* is added as a reference to see how the ratio changes as function of frequency for fixed level of vibration amplitude.

incudomalleolar 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 allowed 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 the 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 (Fig. 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 was seen. Overall, the level of the nonlinearities did not change strongly, but some effect from removing 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 the 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 3D 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 the malleus to stapes would not have any influence on the nonlinearities, 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 nonlinearities, 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 nonlinear than umbo vibrations response. In any case, the results show that the joints between the ossicles do not increase the nonlinearities 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 the umbo and the 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 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 to 70 dB lower than the levels of the vibration amplitudes (see Fig. 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 $1 \mu\text{m}$) in the 1–2-kHz and 4–8-kHz octaves. In the 250–500-Hz octave, detectability is a bit less (about -90 dB re $1 \mu\text{m}$). 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 to 10 dB lower than for the stapes. It is important to realize 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 behavior 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 that at the stapes, it seems logical that more sound pressure is needed for stapes nonlinearity to become detectable. This observation also emphasizes the fact the level at which the nonlinearities become detectable is of little interest in itself.

More important therefore is the way that 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 apparently 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 “linearization” 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 $1 \mu\text{m}$) in the plot for the umbo, we see that this vibration response is reached at 120-dB SPL for a frequency range between 125 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 the vibration response. We find this the most peculiar observation: Apparently, it is not (only) the amplitude of the motion which determines the magnitude of the nonlinearity, as the same amplitude at one frequency results in 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 that we measured is beyond the scope of the current paper and will need a detailed modeling 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), it has been shown that geometric nonlinearity has to be taken into account to obtain correct results (Ladak et al. 2006), and calculations have shown strong asymmetry between displacement patterns found at positive and at negative pressures. In the acoustic range, displacements are far smaller, but response to the negative and positive phase of the sound pressure may still be asymmetric. It is our current hypothesis that membrane geometry is the prime source of this asymmetry and that it may be an important cause of middle ear nonlinear response. We will investigate this in future through a modeling approach.

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.

SUMMARY AND CONCLUSION

In this paper, we measured the vibration response and the nonlinearities of the umbo and the stapes in rabbit ears. Using a very sensitive measurement technique, we were able to detect nonlinearities at an input sound pressure level of 93 dB. In general, we found that nonlinearity as a function of frequency and as a function of sound pressure shows largely the same behavior 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. 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 otoacoustic 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.

ACKNOWLEDGMENTS

The authors wish to thank Fred Wiese and William Deblauwe for their assistance with the measurement setup.

This work was supported by the Research Foundation Flanders—Fonds Wetenschappelijk Onderzoek (FWO).

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

- AERNOUTS J, SOONS J, DIRCKX JJJ (2010) Quantification of tympanic membrane elasticity parameters from in situ point indentation measurements: validation and preliminary study. *Hear Res* 263:177–182. doi:10.1016/j.heares.2009.09.007
- AERTS JRM, DIRCKX JJJ (2010) Nonlinearity in eardrum vibration as a function of frequency and sound pressure. *Hear Res* 263:26–32. doi:10.1016/j.heares.2009.12.022
- BUYTAERT J, VAN DER JEUGHT S, ARS B ET AL (2013) Preliminary human tympanic membrane thickness data from optical coherence tomography. In: Dirckx J, Buytaert J (eds) *Optical measurement techniques for structures & systems²*. Shaker Publishing, Germany, pp 75–84
- DE GREEF D, AERNOUTS J, AERTS J ET AL (2014) Viscoelastic properties of the human tympanic membrane studied with stroboscopic holography and finite element modeling. *Hear Res* 312:69–80. doi:10.1016/j.heares.2014.03.002
- DIRCKX JJJ, DECRAEMER WF (2001) Effect of middle ear components on eardrum quasi-static deformation. *Hear Res* 157:124–137. doi:10.1016/S0378-5955(01)00290-8
- DIRCKX JJJ, BUYTAERT JAN, DECRAEMER WF (2006) Quasi-static transfer function of the rabbit middle ear, measured with a heterodyne interferometer with high-resolution position decoder. *J Assoc Res Otolaryngol* 7:339–351. doi:10.1007/s10162-006-0048-5
- FUNNELL WRJ, KHANNA SM, DECRAEMER WF (1992) On the degree of rigidity of the manubrium in a finite element model of the cat eardrum. *J Acoust Soc Am* 91:2082–2090
- FUNNELL WRJ, HENG SIAH T, MCKEE MD ET AL (2005) On the coupling between the incus and the stapes in the cat. *J Assoc Res Otolaryngol* 6:9–18. doi:10.1007/s10162-004-5016-3
- GOODE RL, KILLION MC, NAKAMURA K, NISHIHARA S (1994) New knowledge about the function of the human middle ear: development of an improved analog model. *Am J Otol* 15:145–154
- GUINAN J, PEAKE W (1967) Middle -ear characteristics of anesthetised cats. *J Acoust Soc Am* 41:1237–1261
- HATO N, STENFELT S, GOODE R (2003) Three-dimensional stapes footplate motion in human temporal bones. *Audiol Neurotol* 8:140–152
- HEFFNER H (1980) Hearing in Glires: domestic rabbit, cotton rat, feral house mouse, and kangaroo rat. *J Acoust Soc Am* 68:1584. doi:10.1121/1.385213
- HUBER A, LINDER T, FERRAZZINI M ET AL (2001) Intraoperative assessment of stapes movement. *Ann Otol Rhinol Laryngol* 110:31–35
- LADAK HM, FUNNELL WRJ, DECRAEMER WF, DIRCKX JJJ (2006) A geometrically nonlinear finite-element model of the cat eardrum. *J Acoust Soc Am* 119:2859. doi:10.1121/1.2188370
- NEDZELNITSKY V (1980) Sound pressures in the basal turn of the cat cochlea. *J Acoust Soc Am* 68:1676–1689. doi:10.1121/1.385200
- PEACOCK J, DIRCKX J, VON UNGE M (2014) Magnetically driven middle ear ossicles with laser vibrometry as a new diagnostic tool to quantify ossicular fixation. *Acta Otolaryngol* 134:352–357. doi:10.3109/00016489.2013.841990
- PINTELON R, SCHOUKENS J (2013) FRF measurement of nonlinear systems operating in closed loop. *IEEE Trans Instrum Meas* 62:1334–1345
- RHODE W (2007) Distortion product otoacoustic emissions and basilar membrane vibration in the 6–9 kHz region of sensitive chinchilla cochlea. *J Acoust Soc Am* 122:2725–2737
- SCHOUKENS J, LATIAIRE J, PINTELON R ET AL (2009) Robustness issues of the equivalent linear representation of a nonlinear system. *IEEE Trans Instrum Meas* 58:1737–1745
- SOONS JAM, AERNOUTS J, DIRCKX JJJ (2010) Elasticity modulus of rabbit middle ear ossicles determined by a novel micro-indentation technique. *Hear Res* 263:33–37. doi:10.1016/j.heares.2009.10.001
- VOSS SE, ROSOWSKI JJ, MERCHANT SN, PEAKE WT (2000) Acoustic responses of the human middle ear. *Hear Res* 150:43–69. doi:10.1016/S0378-5955(00)00177-5
- WILLI UB, FERRAZZINI MA, HUBER AMM (2002) The incudo-malleolar joint and sound transmission losses. *Hear Res* 174:32–44. doi:10.1016/S0378-5955(02)00632-9