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Comparison between distortion product otoacoustic emissions and nerve fiber responses from the basilar papilla of the frog

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The basilar papilla (BP) is one of the three end organs in the frog inner ear that is sensitive to airborne sound. Its anatomy and physiology are unique among all classes of vertebrates. Essentially, the BP functions as a single auditory filter presumably arising from a mechanically-tuned mechanism. As such, both neural and distortion product otoacoustic emission (DPOAE) tuning may reflect a single mechanical filtering mechanism. Using the Duffing oscillator as a simple model for both neural and DPOAE tuning from the BP, two predictions can be made: [1] the characteristic frequency (CF) of neural tuning and the best frequency (BF) of DPOAE tuning will coincide and [2] the neural tuning curve and DPOAE-audiogram have a similar shape when the neural tuning curve is scaled by a factor of 4 along the y-axis. We recorded both neural tuning curves and DPOAE-audiograms from the BP of the leopard frog. These recordings show good agreement with the model predictions when the stimulus tones are related by relatively small stimulus frequency ratios. For larger stimulus frequency ratios, DPOAE recordings clearly deviate from model predictions. These differences are most likely caused by the oversimplified representation of the frog BP by the model. © 2005 Acoustical Society of America. [DOI: 10.1121/1.1871752]

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I. INTRODUCTION

The anatomy of the inner ear exhibits a high degree of variability across the various classes of vertebrates. Notwithstanding this, the transformation of sound pressure into activity in the auditory nerve ultimately involves the same specialized cells in all vertebrates: hair cells.

In frogs, hair cells that are sensitive to airborne sound are distributed over three anatomically separated papillae, each one most sensitive to a different range of frequencies. The sacculus (S) is sensitive to high-level sounds at very low frequencies. No tonotopic organization has been reported for the frog sacculus. The amphibian papilla (AP), which is most sensitive to the low- and mid-frequencies within the frog hearing range, consists of an elongated strip of hair cells. These cells are directly over the rigid wall of the papillar recess, rather than over a flexible membrane (as in the cochlea). Overlying the AP hair cells is a tectorial membrane. Both the shape of this membrane as well as its position within the papillar recess suggest that it plays an important role in the transduction of sound by hair cells (Lewis and Leverenz, 1983). The papilla exhibits tonotopic organization

with the highest (≈ 1.2 kHz) and lowest (≈ 0.1 kHz) frequencies being represented caudally and rostrally, respectively (Lewis *et al.*, 1982).

The third hearing organ in the frog is the basilar papilla (BP). This papilla is most sensitive to the highest frequencies within the hearing range of frogs (Feng *et al.*, 1975). In leopard frogs (*Rana pipiens pipiens*) it responds best to sounds of 1.2–2.4 kHz (Ronken, 1991). The anatomy of the BP is unique among all classes of vertebrates (Wever, 1973); it consists of approximately 50–100 hair cells standing directly over the rigid cartilaginous wall of the papillar recess. Almost all of these hair cells are covered by a tectorial membrane. In an individual animal, almost all BP nerve fibers exhibit very similar tuning curves, with characteristic frequencies (CFs) being essentially identical across nerve fibers (Ronken, 1990; Van Dijk *et al.*, 1997). Also, afferent BP fibers are not suppressible (Feng *et al.*, 1975), and the CF of BP fibers do not depend on temperature (Stiebler and Narins, 1990; Van Dijk *et al.*, 1990). Although it is not completely understood how tuning is achieved in this organ, the unique anatomy and physiology of the BP suggest that tuning relies on the mechanical properties of the structures in the papilla, without the contribution of electrical tuning from the hair cells (Smotherman and Narins, 1999).

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Upon stimulation with two tones that have appropriately chosen frequencies (f_1, f_2) and levels (L_1, L_2), the basilar papilla produces distortion product otoacoustic emissions (DPOAEs; Van Dijk and Manley, 2001). It has been hypothesized that these DPOAEs are the result of the passive non-linear properties of the anatomical structures (i.e., hair cells, the tectorial membrane) found within this organ (Meenderink and Van Dijk, 2004). So, similar to the nerve, the tuning of DPOAEs seems to be the result of a mechanically tuned filter. Thus, both neural and DPOAE tuning may reflect the same, single auditory filter, with the tuning having a mechanical origin.

Recently, the Duffing oscillator has been used as a simple model for DPOAEs from the BP (Van Dijk and Manley, 2001; Meenderink *et al.*, 2005). These studies show that such a simple model qualitatively describes the dependence of emission amplitude and phase on both absolute (f_1, f_2) and relative (f_2/f_1) stimulus frequencies. In the present study we used the Duffing oscillator as a model for both DPOAEs and neural tuning from the BP. We show that in the model (1) the CF of the frequency threshold curve and the stimulus frequency resulting in a maximum distortion product amplitude coincide. This correlation is independent of the ratio f_2/f_1 used to evoke distortion products; and (2) the shapes of the frequency tuning curves and the DP audiograms are markedly different: the frequency tuning curves are much broader than the DP audiograms. However, when the frequency tuning curves are scaled by a factor of 4 along the y axis, the two curves become almost identical in shape.

We tested these two model predictions by obtaining both neural tuning curves and several DPOAE audiograms from the basilar papilla of the leopard frog *R. pipiens pipiens*. When using relatively small stimulus frequency ratios ($f_2/f_1 < 1.16$) during DPOAE recordings, the first model prediction is found. Also, the neural tuning curves are much more broadly tuned than DPOAE audiograms. Scaling of the neural tuning curves by a factor of 4 does render the two curves more similar, although it never results in identically shaped curves. Probably, the theoretical scaling factor of 4 is not applicable to the frog ear, which is most likely caused by the oversimplified representation of the frog BP in the used model.

II. METHODS

A. Animal care

Recordings were made from the left ear of the Northern leopard frog *Rana pipiens pipiens*: $n=9$; body mass: 17.7–31.9 g (mean 24.7 g); snout-vent length: 58.6–78.4 mm (mean: 67.9 mm). Due to the duration of the experiments, nerve fiber recordings were performed the day following the DPOAE recordings. Prior to both DPOAE and nerve fiber recordings the animal was anesthetized by intramuscular injection of sodium pentobarbital (Nembutal: 60 mg/ml; 1.0 μ l/g body mass) in one of the hind limbs. If necessary, supplementary anesthesia was administered during the experiments. Recordings were performed inside a sound-attenuating room, with the frog placed on a vibration-isolation table. To prevent dehydration and to facilitate

cutaneous respiration during the experiments, the animal was covered by gauze soaked in frog Ringer's solution. At the end of the nerve-fiber experiment, animals were killed by destruction of the central nervous system (double-pith procedure). The "Principles of Care" (NIH publication 85-32, revised 1985) and USA regulations were followed throughout this study, and protocols were approved by the University of California Animal Research Committee.

B. DPOAE recordings

Distortion product otoacoustic emissions (DPOAEs) were recorded using a setup and method described previously (Meenderink *et al.*, 2005). Briefly, after anesthetizing the animal, the open end of a custom-built probe was sealed to the skin surrounding the tympanic membrane using silicone grease. The probe contained two miniature speakers (ER-10C, Etymotic Research) for stimulus delivery and one 1/2 in. condenser microphone (Brüel & Kjær type 4134), connected to a preamplifier (B&K type 2069; 60 dB amplification), for emission recording. In order to evoke DPOAEs, two stimulus tones with frequencies f_1 and f_2 (where $f_1 < f_2$) and levels L_1 and L_2 were played simultaneously, one from each speaker. All DPOAE recordings were made with stimulus levels $L_1 = L_2 = 76$ dB SPL. The stimulus frequency f_1 was systematically increased from 213 to 2774 Hz in approximately 30 Hz steps. This f_1 sweep was repeated 11 times, each sweep with the frequency ratio f_2/f_1 fixed at a different value (f_2/f_1 varied from 1.02 to 1.4, respectively).

The generation of the stimulus tones, as well as the recording of the microphone signal, was controlled by a desktop computer, using software written in Matlab (The Mathworks, Inc.) and Rpvds (Tucker Davis Technologies). This software controlled two separate D/A channels (RP2,TDT) and two programmable attenuators (PA5,TDT) for stimulus generation, and an A/D channel (RP2,TDT) used for recording of the microphone signal.

The two stimulus frequencies were chosen such that they were periodic over the same integer number of sample points. This ensures that the DPOAE frequencies are also periodic over that same number of sample points, resulting in sample blocks in which all frequencies of interest (f_1 , f_2 , and f_{DPOAE}) are periodic. For each stimulus frequency pair, f_1 , f_2 , a series of these blocks was recorded. After artifact rejection and averaging of these blocks the levels of the stimulus tones and the DPOAEs were calculated by Fourier analysis.

C. Nerve fiber recordings

The day after the DPOAE recordings, the frog was re-anesthetized (using the same dosage of anesthesia as during the DPOAE recordings) in preparation for the recordings from the VIIIth nerve. Before the animal was placed inside the sound-attenuating room, the auditory nerve that innervates the left ear was surgically exposed using a ventral approach (Capranica and Moffat, 1975; Zelick and Narins, 1985). The frog was placed on its back and the lower jaw was retracted to expose the roof of the mouth. After covering the frog with wet gauze, a small hole was drilled on the

lateral side of the brain case, thus exposing the VIIIth nerve and its point of entry into the brain. After surgery, the animal was transferred to the sound-attenuating room to conduct the experiments. For the intracellular recordings from individual nerve fibers, we used conventional glass electrodes filled with 3 M KCL (resulting in an impedance typically >40 M Ω). Under visual control, the tip of the electrode was placed over the surface of the auditory nerve and advanced into the nerve using a remotely driven hydraulic microdrive (Narishige MO-103L). The signal from the electrode was fed through a high input impedance preamplifier (Winston Electronics, Model 1000) and a spike analyzer (Mentor N-750) before being digitized using an A/D converter (DD1,TDT).

Pure-tone stimuli (0.3–5.0 kHz) were generated from a D/A converter (DD1,TDT), set to the correct level using a programmable attenuator (PA4,TDT) and presented to the operated ear through a dynamic speaker (Beyer DT48). This speaker was coupled to the frog ear via a brass housing and a short piece of rubber tubing. A tight seal between the tubing and the skin surrounding the tympanic membrane was obtained using silicone grease. The brass housing also contained a 1/2 in. condenser microphone (Brüel & Kjær type 4134), which was used to calibrate the level of the stimulus tone.

While advancing the electrode through the nerve using the microdrive, the ear was stimulated using short bursts of white noise. As soon as a nerve fiber was encountered, an automated staircase algorithm was used to obtain two frequency threshold curves (FTCs) using an “up–down” threshold-tracking method (Evans, 1979): once from low to high frequencies, and once from high to low frequencies. The automated system consisted of software running on a desktop computer and controlled both stimulus generation and data acquisition. The two FTCs were recorded with a frequency resolution of 40 points/octave and a 1 dB intensity resolution. Their average was calculated and subsequently smoothed using a moving Hanning window (window width = $1/8$ octave).

III. THEORY: THE DUFFING OSCILLATOR

As a simple model for the basilar papilla, we will consider the Duffing oscillator. This oscillator has been used previously to model distortion product otoacoustic emissions from the BP (Van Dijk and Manley, 2001; Meenderink *et al.*, 2005). Although the Duffing oscillator is an (over)simplification of the basilar papilla mechanics, it models remarkably well the relation between the amplitude and phase of the DPOAE, and the stimulus frequencies f_1 and f_2 (Meenderink *et al.*, 2005). The Duffing oscillator is described by the second-order nonlinear equation,

$$m\ddot{x} + R\dot{x} + k(x)x = F(t), \quad (1)$$

where m is a mass, whose movement is driven by the force $F(t)$. This movement is impeded by both a resistance R , and a nonlinear stiffness $k(x) = k_0(1 + x^2/x_0^2)$.

In order to evoke distortion products, the oscillator is driven by a two-tone force,

$$F(t) = A_1 \sin(2\pi f_1 t) + A_2 \sin(2\pi f_2 t), \quad (2)$$

FIG. 1. Tuning properties for distortion products and nerve fiber from a Duffing oscillator model [see Eq. (1)]. Results are for $m=1$, $r=1$, $k_0 = 4\pi^2$, $x_0=1$, and $A_1=A_2=1$. (a) The DPOAE amplitude for $2f_1 - f_2$ is plotted as a function of f_1 . Each curve displays a DP audiogram obtained with a different f_2/f_1 . For clarity, the curves are offset by 20 dB, with $f_2/f_1 = 1.02$ being at the bottom. Successive curves represent data obtained with increasing f_2/f_1 , with the curve for $f_2/f_1 = 1.4$ at the top. The maximum in each DP audiogram is indicated by the filled circles. (b) The same as (a), but for $2f_2 - f_1$ plotted as a function of f_2 . Each DP audiogram has its maximum when stimulus frequency f_1 (for $2f_1 - f_2$) or f_2 (for $2f_2 - f_1$) coincides with the resonant frequency of the Duffing oscillator. (c) FTC (black, dashed line) as a function of stimulus frequency. The characteristic frequency (CF) of this curve coincides with the resonant frequency of the oscillator, and as a consequence also with the maximum in the DP audiograms. For comparison, a single DP audiogram ($2f_1 - f_2$, $f_2/f_1 = 1.02$) is also shown, but with its y axis reversed (black, solid line). It can be seen that the tuning of the FTC is much broader than the tuning of the DP audiogram. This difference is absent when the FTC is scaled by a factor of 4 along the y axis (gray, dashed line). To visually distinguish the DP audiogram from the scaled FTC, the latter is plotted with a small vertical offset.

where A_1 and A_2 are the amplitudes of the two tones at frequencies f_1 and f_2 ($f_2 > f_1$), respectively. We calculated distortion products for a range of two-tone combinations: f_1 varied between 0.2 and 2.0 in 0.01 steps, with the normalized resonant frequency being 1, while f_2/f_1 was between 1.02 and 1.4 in steps of 0.01. Figures 1(a), 1(b) show DP amplitude when $m=1$, $r=1$, $k_0 = 4\pi^2$, $x_0=1$, and $A_1=A_2=1$. Here, individual lines give DP amplitude, obtained with a fixed- f_2/f_1 , as function of stimulus frequency (either f_1 for $2f_1 - f_2$, or f_2 for $2f_2 - f_1$). It can be seen that for $2f_1 - f_2$ [Fig. 1(a)], each DP audiogram has its maximum when f_1 coincides with the resonant frequency of the oscillator, as indicated by the filled circles. In contrast, for $2f_2 - f_1$ [Fig. 1(b)], maxima in the different DP audiograms occur when f_2

coincides with the oscillator's resonant frequency (filled circles). Notice that the DP audiograms obtained with relatively large f_2/f_1 exhibit three more or less pronounced lobes, rather than only one single relative maximum. These lobes arise whenever f_1 , f_2 , or f_{DP} coincide with the resonant frequency of the oscillator, but merge into a single maximum when f_2/f_1 is relatively small.

The Duffing oscillator was also used to model frequency threshold curves (FTCs) in the basilar papilla. A FTC is a function that specifies for each frequency f the corresponding amplitude A required to reach a threshold response in the nerve fiber. Typically, the threshold is defined as an increase in the number of spikes above the spontaneous firing that occurs during a short interval of time. Rather than adding some spike-generator to the model, we will assume that the FTC is inversely proportional to the frequency-response curve of the Duffing oscillator, as expressed by the movement x [Eq. (1)]. This will be approximately true for relatively low stimulus levels, when the growth of the response (i.e., spike rate) with increasing stimulus level is (approximately) linear. The inverted frequency-response function for the Duffing oscillator is shown in Fig. 1(c) (black dashed line) for the same parameters used for the generation of distortion products. The response curve was computed by driving the oscillator by a fixed-amplitude, one-tone force,

$$F(t) = A \sin(2\pi ft), \quad (3)$$

where A and f are the amplitude and the frequency of the stimulus, respectively. The characteristic frequency (CF, i.e., the stimulus frequency that requires the lowest stimulus level A for which movement x reaches the threshold) of the FTC coincides with the resonant frequency ($f=1$) of the oscillator.

In addition to the FTC, Fig. 1(c) also shows a DP audiogram (black solid line; $2f_1-f_2$ obtained with $f_2/f_1=1.02$). To facilitate a graphical comparison between the two, the DP audiogram is plotted with a reversed y axis (the left y axis corresponds to the DP audiogram). The FTC is plotted over the same dB range (right y axis) as the DP audiogram, with its offset chosen to match the minima of the two curves. This shows that the FTC is much more broadly tuned than the DP audiogram. However, when the FTC is scaled by a factor of 4 along the y axis (gray dashed line), its shape becomes almost identical to that of the DP audiogram.

The necessity to scale the FTC by a factor of 4 to make its shape identical to the shape of the inverted DP audiogram can be explained by considering the following example. For a given combination of stimulus tones, let us change stimulus frequency f_1 in such way that the response of the oscillator [as expressed in x in Eq. (1)] to this stimulus tone increases by 1 dB. But this shift of f_1 will also result in a shift of stimulus frequency f_2 . When using a small stimulus frequency ratio f_2/f_1 , this frequency shift of f_2 will be approximately the same as the shift for f_1 . At the same time, the small ratio f_2/f_1 ensures that f_1 and f_2 change over (approximately) the same absolute frequencies. As a consequence, the change in the movement x in response to the f_2 frequency shift will be similar to the change induced by the f_1 frequency shift (i.e., 1 dB). For a power-law nonlinearity,

such a 1 dB increase in both of the stimulus tones will result in a distortion product that is increased in amplitude by 3 dB. This alone would give rise to a scaling factor of 3. But the frequency shift of both stimulus tones results in a frequency shift of the DPOAEs as well. Since the generated DPOAEs themselves are part of the response x , they will be filtered accordingly. This filtering, resulting from the frequency shift, also changes the DPOAE amplitude by about 1 dB. Combined, the 3 dB increase (caused by the 1 dB increase from the shift of the stimulus frequencies) and the 1 dB increase (due to the change in DPOAE frequency) result in a scaling factor of 4.

In summary, using the Duffing oscillator as a model for the basilar papilla reveals that the maximum DP amplitude occurs when one of the stimulus tones (f_1 for $2f_1-f_2$ and f_2 for $2f_2-f_1$, respectively) coincides with the CF of the FTC. This is independent of the stimulus frequency ratio f_2/f_1 used. Also, the FTC is much more broadly tuned than the DP audiogram, but this difference is absent when the FTC is scaled by a factor of 4 along the y axis.

IV. RESULTS

Distortion product otoacoustic emissions (DPOAEs) were obtained for a range of stimulus frequencies ($213 < f_1 < 2774$ Hz). As a consequence, DPOAEs were recorded from both the amphibian papilla (AP) and the basilar papilla (BP). It is currently believed that the generation of DPOAEs from both papillae gives rise to the bimodal frequency dependence of emission amplitude in DPOAE audiograms: in the leopard frog, DPOAEs evoked with stimulus frequencies below 1250 Hz presumably arise from the AP, while DPOAEs evoked with higher stimulus frequencies are thought to arise from the BP. Following Van Dijk and Manley (2001), we will refer to emissions from the amphibian papilla as AP-DPOAEs, and those from the basilar papilla as BP-DPOAEs.

The frequency dependence of emission amplitude can be seen in Fig. 2, which shows a series of DPOAE audiograms, each one obtained with a different stimulus frequency ratio f_2/f_1 . A relative minimum in emission amplitude is found when either f_1 [for $2f_1-f_2$, Fig. 2(a)] or f_2 [for $2f_2-f_1$, Fig. 2(b)] is approximately 1250 Hz. Both for lower and higher stimulus frequencies, emission amplitude exhibits a relative maximum. The filled circles indicate the maximum DPOAE in the basilar papilla frequency range (stimulus frequencies > 1250 Hz) in each DPOAE audiogram. For increasingly larger stimulus frequency ratios f_2/f_1 (successive lines in Fig. 2) it can be seen that this maximum occurs for a fixed stimulus frequency (fixed f_1 for $2f_1-f_2$ and fixed f_2 for $2f_2-f_1$, respectively). Only for the largest stimulus frequency ratios, the maximum BP-DPOAE is found for somewhat lower stimulus frequencies. In the following, we will refer to the stimulus frequency (f_1 for $2f_1-f_2$ and f_2 for $2f_2-f_1$) that results in the maximum BP-DPOAE as the distortion product best frequency BF_{DP} . The term best frequency (BF) is used, rather than characteristic frequency (CF), since DPOAE audiograms are equal-input curves. This in contrast to FTCs, which are equal-output curves and hence result in characteristic frequencies.

FIG. 2. A series of DPOAE audiograms for (a) $2f_1-f_2$ and (b) $2f_2-f_1$ obtained in one frog. Each DPOAE audiogram was recorded by varying the two stimulus tones while keeping the stimulus frequency ratio constant (i.e., fixed f_2/f_1 sweeps). For clarity, curves are offset by 10 dB with $f_2/f_1 = 1.02$ at the bottom and $f_2/f_1 = 1.4$ at the top. The stimulus levels were fixed and constant in all sweeps ($L_1=L_2=76$ dB SPL). Notice that for $2f_1-f_2$ the stimulus frequency f_1 is on the abscissa, while for $2f_2-f_1$ the stimulus frequency f_2 is on the abscissa. All DPOAEs show a bimodal dependence on frequency, with a single relative maximum for frequencies above and below 1250 Hz, respectively. It is currently believed that this reflects DPOAEs from both the amphibian papilla (AP-DPOAE, $f < 1250$ Hz) and basilar papilla (BP-DPOAE, $f > 1250$ Hz). For the BP-DPOAE, maxima in emission amplitudes are indicated by the filled circles.

The dependence of BF_{DP} on stimulus frequency ratio f_2/f_1 is illustrated in Fig. 3. This figure shows the shift in BF_{DP} (the shift is relative to BF_{DP} for $f_2/f_1 = 1.02$) as a function of stimulus frequency ratio f_2/f_1 , averaged across all frogs. Both for DPOAE at $2f_1-f_2$ (“+,” solid line) and $2f_2-f_1$ (“○,” dashed line), BF_{DP} remains unchanged for relatively small frequency ratios ($f_2/f_1 < 1.16$). For increasingly larger ratios, BF_{DP} shifts to increasingly lower frequencies.

FIG. 3. The stimulus frequency that resulted in the maximum BP-DPOAE (BF_{DP}) as a function of the stimulus frequency ratio f_2/f_1 , averaged over all frogs. The BF_{DP} found at $f_2/f_1 = 1.02$ is taken as a reference, and changes in BF_{DP} are expressed relative to this reference. For neither $2f_1-f_2$ (“+,” solid line) nor $2f_2-f_1$ (“○,” dashed line) does BF_{DP} change for relatively small stimulus frequency ratios ($f_2/f_1 < 1.16$). For larger ratios, BF_{DP} shifts toward lower frequencies in both DPOAEs.

FIG. 4. Scatter plot of the stimulus frequency resulting in the maximum BP-DPOAE amplitude (BF_{DP}) for $f_2/f_1 = 1.02$ versus the characteristic frequency (CF_{nerve}) of the nerve fiber FTC. For $2f_1-f_2$ (filled “□”) BF_{DP} refers to stimulus frequency f_1 , while for $2f_2-f_1$ (“○”) BF_{DP} refers to stimulus frequency f_2 . As a reference, the diagonal dashed line indicates the condition for which $BF_{DP} = CF_{nerve}$.

Besides BP-DPOAEs, we also obtained frequency threshold curves (FTCs) from each frog. To ascertain that a FTC was obtained from a nerve fiber that innervated the basilar papilla, not only CF and the corresponding threshold level (CL) were determined, but also the bandwidth of the tuning curve at 10 dB above CL (i.e., W_{10}). Ronken (1991) showed that W_{10} can be used to discriminate between nerve fibers innervating the AP and the BP, respectively. In our results, nerve fibers that qualified as BP units are those for which W_{10} ranged from 1272 to 2161 Hz, while CFs were between 1464 and 1949 Hz. Besides FTCs from basilar papillar fibers, we also made recordings from nerve fibers that innervated the amphibian papilla and the sacculus, respectively. For the present paper, these recordings were ignored. To distinguish the FTC tuning properties from those calculated for BP-DPOAE, we will add the subscript “nerve” to them.

A graphical comparison between the tuning properties of the FTCs and the DPOAE audiograms (i.e., CF vs BF and a comparison between CL, W_{10} , and $Q_{10} = CF/W_{10}$, respectively) revealed no clear quantitative relation between the two, except when CF and BF were compared (Fig. 4). This figure shows BF_{DP} from DPOAE audiograms obtained with $f_2/f_1 = 1.02$ against CF_{nerve} from the FTCs, combined for all frogs. Both for DPOAE at $2f_1-f_2$ (filled “□”) and $2f_2-f_1$ (“○”) the BF_{DP} roughly coincides with the CF_{nerve} of the accompanying FTC.

Figure 5 shows three examples of $2f_1-f_2$ DPOAE audiograms obtained with $f_2/f_1 = 1.02$ (black, solid line) and FTCs (black, dashed line). Each subplot shows data obtained in one frog. To facilitate a graphical comparison with the FTC, the DPOAE audiogram is plotted with a reversed y axis (the left y axis corresponds to the DPOAE amplitude), while the x axis displays stimulus frequency f_1 . The stimulus level range for the FTC (right y axis) was chosen to match the amplitude range used to display the DPOAE audiogram, while the offset was chosen to match the CF of the FTC and the BF of the (reversed) DPOAE audiogram. This is the

FIG. 5. Examples of FTCs (black; dashed line) and DPOAE audiograms for $2f_1 - f_2$ (black, solid line) recorded in three different frogs. DPOAEs were obtained with $f_2/f_1 = 1.02$. In each panel, the stimulus frequency f_1 for the DPOAE audiograms is indicated on the abscissa, while the left y axis shows the DPOAE amplitude. Notice that this axis goes from high to low levels, resulting in a reversed representation of the DPOAE audiogram. The right y axis shows the threshold stimulus levels for the FTC. The range of both y axes are identical, while the offsets were chosen to match the minima of both curves. In each panel, the gray dashed line is the same FTC, but with its y axis scaled by a factor of 4.

same representation as was used in Fig. 1(c). All three subplots show that the CF_{nerve} and the BF_{DP} are almost identical, as was already illustrated in Fig. 4. For frequencies away from CF, the two curves deviate from each other, resulting in a much broader tuning of the FTC compared to the BP-DPOAE. However, when the FTC is scaled by a factor of 4 along its y axis (gray dashed lines), the shape of the FTC and the BP-DPOAE become more similar. For frequencies above CF, the two curves either nearly overlap [Figs. 5(a), 5(b)], or are oriented approximately parallel [Fig. 5(c)]. For frequencies below CF, the shape of the FTC and the BP-DPOAE also display similarities. For instance, in the BP-DPOAE of Fig. 5(a), the relative minimum near 1300 Hz coincides with relatively large DPOAEs at the same stimulus frequency f_1 . In Figs. 5(b), 5(c) the correspondence between the two curves disappears below about 1500 Hz, presumably due to the contribution of distortion products from the AP.

V. DISCUSSION

In the present study, we reported on both distortion product otoacoustic emissions (DPOAEs) and nerve fiber frequency threshold curves (FTCs) obtained from the frog

basilar papilla (BP). We obtained DPOAEs for a range of stimulus frequencies ($213 < f_1 < 2774$ Hz). This range covers frequencies of the highest sensitivity in both the amphibian papilla (AP) and the basilar papilla (BP). It is currently believed that emission-generation in both papillae results in the bimodal frequency dependence in the DPOAE audiograms (Van Dijk and Manley, 2001; Van Dijk *et al.*, 2002; Meenderink and Van Dijk, 2004). This is based on the idea that relative maxima in emission amplitudes arise when the stimulus maximally excites either the AP or the BP. For submaximal stimulation of the papillae, the emission amplitude will be relatively reduced. So, the frequency separation between the two maxima in the DPOAE audiogram would depend on the frequency separation of the highest sensitivity of the AP and the BP. In ranid frogs, the AP gives rise to fibers tuned to frequencies up to 1.5 kHz, while the tuning of the BP varies with frog species. This would result in a species-specific frequency separation of the two relative maxima in the DPOAE audiogram, as is indeed found. For instance, in *Hyla cinerea*, the BP is most sensitive to frequencies of approximately 3.5 kHz (Capranica and Moffat, 1983) and the two relative maxima in the DPOAE audiogram from this species are separated by a frequency range in which no emissions are detected (Van Dijk and Manley, 2001). In the leopard frog, the BP is tuned to lower frequencies, partially overlapping with the frequency range covered by the AP (Ronken, 1991). As a result, DPOAE audiograms show relatively large DPOAE amplitudes in the frequency ranges 500–1200 Hz and 1300–2500 Hz, corresponding to the frequency ranges of the highest sensitivity of the amphibian and the basilar papilla, respectively. In *R. pipiens pipiens* an amplitude notch is often observed in between these two frequency ranges at about 1250 Hz. This suggests that near that frequency, emissions from both papillae may interfere. However, these results strongly support the notion that below and above the notch the DPOAE signal is dominated by a component from either the amphibian or the basilar papilla, respectively.

In the model, DP audiograms obtained with relatively large f_2/f_1 exhibit three more or less pronounced lobes, rather than only a single maximum (Fig. 1). These arise whenever f_1 , f_2 , or f_{DP} coincides with the resonant frequency of the Duffing oscillator. These three lobes are not immediately apparent in our BP-DPOAE data. Their absence is most likely caused by the relatively broad tuning of the basilar papilla. As a consequence, the frequency ratios f_2/f_1 we used did not result in a frequency separation between the two stimulus tones large enough to separate the three lobes in the DPOAE audiogram. This is similar to why the DP audiograms, obtained with small f_2/f_1 , from the model exhibit only a single maximum. The frequency separation between the stimulus tones in relation to the tuning width of the filter is insufficient to separate the three lobes.

The BP is of interest to hearing research, since it functions as a single auditory filter (Ronken, 1990; Van Dijk and Manley, 2001). This property is not found in any other vertebrate hearing organ. In addition, the tuning properties of nerve fiber FTCs and DPOAEs probably are different manifestations of the same mechanical tuning mechanism present

in this papilla, as is implied by the following two considerations (Van Dijk and Manley, 2001): (1) neural tuning of nerve fibers that innervate the basilar papilla presumably originates from a mechanical tuning mechanism (Smotherman and Narins, 1999). Since this papilla lacks a basilar membrane, or an analog structure (hair cells are embedded in the rigid wall of the papillar recess), it is reasonable to assume that the mechanical tuning is the result of the mechanics of the hair bundles and the tectorial membrane; (2) BP-DPOAEs are probably generated by the nonlinear mechanics associated with the basilar papilla hair cells and their overlying tectorial membrane (Meenderink and Van Dijk, 2004). Combining these two considerations, one may conclude that the BP-DPOAEs and the neural FTC very likely reflect the same mechanical filter.

Recently, the Duffing oscillator has been used as a model for DPOAEs from the basilar papilla (Van Dijk and Manley, 2001; Meenderink *et al.*, 2005). These studies show that such a simple model qualitatively describes the dependence of emission amplitude and phase on both absolute (f_1, f_2) and relative (f_2/f_1) stimulus frequencies. Since tuning in nerve fibers from the basilar papilla arises from the same mechanism as the DPOAE, we also used the Duffing oscillator to model nerve fiber frequency threshold curves. These calculations showed several correlations between threshold curves and the tuning properties of the distortion products: (1) distortion product amplitude is maximized whenever the stimulus frequency that is closest to the distortion product frequency (i.e., f_1 for $2f_1 - f_2$ and f_2 for $2f_2 - f_1$) coincides with the resonant frequency of the oscillator. This is independent of the stimulus frequency ratio f_2/f_1 used [Figs. 1(a), 1(b)]. Also, the characteristic frequency (CF) of the FTC also coincides with the resonant frequency of the oscillator [Fig. 1(c)]. Thus, $CF_{\text{nerve}} = BF_{\text{DP}}$, independent of the ratio f_2/f_1 used to obtain the DP audiogram. (2) The low- and high-frequency slopes of the DP audiogram are much steeper than those of the FTC. As a consequence, the FTC is much more broadly tuned than the DP audiogram. In the model, this difference in the shape/slope of the two functions is almost absent when the FTC is scaled by a factor of 4 along the y axis [Fig. 1(c)]. We will discuss these two model predictions in relation to the BP-DPOAEs and FTCs obtained in the frog.

In the simple model, the FTC is inversely proportional to the amplitude response. Thus, CF_{nerve} will equal the resonant frequency of the model oscillator. At the same time, the DP amplitude is maximized when either f_1 (for $2f_1 - f_2$) or f_2 (for $2f_2 - f_1$) coincides with the resonant frequency. The combination of these two results gives that $BF_{\text{DP}} = CF_{\text{nerve}}$, independent of the stimulus frequency ratio f_2/f_1 . The scatter plot in Fig. 4 shows that in the frog $BF_{\text{DP}} = CF_{\text{nerve}}$ for $f_2/f_1 = 1.02$. As expected from the model, this relation is maintained for increasing f_2/f_1 (as long as $f_2/f_1 < 1.16$), since BF_{DP} does not change when the stimulus frequency ratio is increased (Fig. 3). However, for larger f_2/f_1 the maxima in the DPOAE audiograms occur for lower stimulus frequencies, i.e., BF_{DP} decreases, and the predicted relation between BF_{DP} and CF_{nerve} is no longer found. This discrepancy

between the results and the model may be understood when considering the simplifications made by using the Duffing oscillator as a model for the BP.

The Duffing oscillator is intended to model the mechanics of the tectorial membrane and the hair cell stereovilli of the basilar papilla. The oscillator is a simplification of this system in two respects. First, the Duffing oscillator is a second-order system, containing one stiffness element and one mass. In contrast, the basilar papilla contains between 50–100 hair cells, and the tectorial membrane is a flexible structure with multiple degrees of freedom. This structure presumably behaves as a high-order system, as is confirmed by recordings from primary auditory nerve fibers (Narins and Hillery, 1983; Van Dijk *et al.*, 1994). Second, input–output functions of DPOAEs from the frog ear (Meenderink and Van Dijk, 2004) show that the nonlinear element cannot be as simple as the parabolic stiffness of the Duffing oscillator [Eq. (1)].

The second result from the model is that the filter shapes of the FTC and the DP audiogram are quite different, with the former much more broadly tuned than the latter. In the model, this difference is largely absent when the FTC is scaled by a factor of 4 along the y axis.

In qualitative agreement with the model, neural tuning curves are more broadly tuned than DPOAE audiograms. From Fig. 5 it can be seen that the application of a scaling factor of 4 to the recorded FTCs “improves” the correspondence between the FTC and the DPOAE audiogram. In the first case [Fig. 5(a)] the scaled FTC closely follows the DPOAE audiogram for frequencies above 1100 Hz. In the second case [Fig. 5(b)] only the tip and the high-frequency slope of the scaled FTC and the DPOAE audiogram coincide. Finally, in the third case [Fig. 5(c)] the tip and part of the low-frequency slopes of both curves coincide.

For frequencies below 1250 Hz, the discrepancy between the (scaled) FTC and the DPOAE audiogram suddenly becomes quite pronounced. This presumably originates from the contribution of the amphibian papilla to the recorded emission signal below this frequency.

The application of a scaling factor of 4 was motivated by the model results. The model includes a cubic nonlinearity, the Duffing stiffness term. Although this may appear to be an expansive nonlinear term, it is actually a compressive nonlinear model: with increasing stimulus level, the stiffness and, consequently, the total impedance of the model rapidly increases.¹ However, we considered the response of the Duffing oscillator at small stimulus amplitudes. Then, the growth of the distortion products is dominated by the cubic nonlinearity, and for fixed stimulus frequencies the DP grows at 3 dB/dB. When simulating FTCs and DP audiograms, the stimulus frequencies are not fixed. As argued in Sec. III, the distortion product itself is also subject to the second-order bandpass character of the oscillator. Consequently, the use of the Duffing oscillator requires a scaling factor of 4.

In frogs, the growth rate of DPOAEs from the BP is close to 2 dB/dB, rather than 3 dB/dB, over a large range of stimulus levels. Only at moderate stimulus intensity, emission levels grow less steep with stimulus amplitude (Meenderink and Van Dijk, 2004). In line with the argument given

in Sec. III, it would be expected that FTCs, scaled by a factor of 3 (i.e. $2+1$), would overlap with the DPOAE audiograms. Obviously, both the model and the data presented are not consistent with this expectation. From the rather complex shape of DPOAE input/output functions (Meenderink and Van Dijk, 2004) it follows that the characteristic of the nonlinearity in the ear must be more complex than a simple cubic stiffness term. This would result in a relation between FTCs and DPOAE audiograms that is more complex than described by the Duffing oscillator, also rendering the derivation of the scaling factor more difficult.

Finally, the usefulness of a comparison between details in the shape of a FTC and a DPOAE audiogram is limited by the fact that the former is an iso-output measure and the latter is an iso-input measure. Obtaining both neural and emission responses at a range of input levels may allow for a better comparison between the two quantities.

VI. CONCLUSIONS

We presented both DPOAEs and nerve fiber tuning data from the frog basilar papilla and compared their properties. This comparison is of interest because the BP not only functions as a single auditory filter (Ronken, 1990; Van Dijk and Manley, 2001), it is also believed that both the tuning of nerve fibers (FTC) and BP-DPOAEs are the result of the same mechanical filter (Van Dijk and Manley, 2001). As a simple model for such a single auditory filter we used the Duffing oscillator. This model predicts two correlations between the tuning of DPOAEs and neural tuning. (1) The characteristic frequency of the FTC and the best frequency of BP-DPOAEs will coincide ($CF_{\text{nerve}} = BF_{\text{DP}}$), independent of the ratio f_2/f_1 used during DPOAE recordings, and (2) the shape of the FTC and the BP-DPOAE will be quite different, with the FTC much more broadly tuned compared to the BP-DPOAE. In the model, this difference largely disappears when the FTC is scaled by a factor of 4 along the y axis.

To some degree, the results obtained from the frog basilar papilla qualitatively agree with these model predictions. For small stimulus frequency ratios f_2/f_1 , the CF_{nerve} and the BF_{DP} were similar. Only for larger f_2/f_1 , the first model prediction was no longer found, most likely caused by the oversimplified representation of the BP mechanics in the model. Also in line with model predictions, the shape of the FTC and the DPOAE audiogram were quite different, with the former being much broader tuned than the latter.

In the model, the FTC and the DP audiogram match each other after scaling the FTC by a factor of 4. In the basilar papilla, this scaling procedure results in a partial overlap of these curves. The differences between the scaled FTC and the DPOAE audiogram are not too surprising. The derivation of the scaling factor depends on the nonlinear characteristics of the oscillator. In the model, the relatively small stimulus levels result in a constant scaling factor of 4. In the frog, it seems that the complex nonlinear behavior of the basilar papilla will result in a scaling factor that is not constant, but one that will vary depending on the parameters of the two stimulus tones.

We conclude that the single oscillator model, in which emission and neural tuning result from the same filter, quali-

tatively describes the behavior of the frog basilar papilla. This supports the idea that this hearing organ functions as a single auditory filter.

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¹Note that when considering only the nonlinear terms on the left-hand side of Eq. (1), it becomes $x^3/x_0^2 = F(t)$. Thus, with $F(t)$ being the "input" and $x(t)$ the "output," the input/output function is compressive: $x(t) = F^{1/3}(t)x_0^{2/3}$. Although this is an oversimplification of the oscillator, it illustrates the oscillator's compressive behavior.

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