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Level dependence of distortion product otoacoustic emissions in the leopard frog, *Rana pipiens pipiens*

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Abstract

The inner ear of frogs holds two papillae specialized in detecting airborne sound, the amphibian papilla (AP) and the basilar papilla (BP). We measured input–output (I/O) curves of distortion product otoacoustic emissions (DPOAEs) from both papillae, and compared their properties. As in other vertebrates, DPOAE I/O curves showed two distinct segments, separated by a notch or kneepoint. The slope of the low-level segment was conspicuously different between the AP and the BP. For DPOAE I/O curves from the AP, slopes were ≤ 1 dB/dB, similar to what is found in mammals, birds and some lizards. For DPOAE I/O curves from the BP these slopes were much steeper (≈ 2 dB/dB). Slopes found at high stimulus levels were similar in the AP and the BP (≈ 2 dB/dB). This quantitative difference between the low-level slopes for DPOAEs from the AP and the BP may signify the involvement of different mechanisms in low-level DPOAE generation for the two papillae, respectively.

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Keywords: Distortion product otoacoustic emissions; Input–output curves; Amphibia; Frog

1. Introduction

When the ear is stimulated with two primary tones with appropriately chosen frequencies (f_1, f_2) and levels (L_1, L_2), distortion product otoacoustic emissions (DPOAEs) can be recorded outside the ear. DPOAEs have been reported in mammals (e.g. Kemp, 1979), birds (Taschenberger and Manley, 1998), reptiles (Manley et al., 1993), amphibia (Van Dijk and Manley, 2001), and insects (Coro and Kössl, 2001). They are a manifestation of the nonlinear mechanisms involved in the processing of acoustic stimuli in the inner ear.

Across species, DPOAE exhibit several similarities. In small mammals, reptiles and insects, slopes of input–output (I/O) curves for DPOAE at $2f_1 - f_2$ depend on the levels of the stimulus tones (mammals: Brown, 1987; reptiles: Manley et al., 1993; insects: Coro and Kössl, 2001). For low- and high-level stimulus levels, DPOAE amplitude grows with a relatively constant slope with increasing stimulus levels. For intermediate stimulus levels, these slopes are decreased, resulting in either a kneepoint (slope remains positive) or a notch (slope becomes negative) in the I/O curves. The DPOAE I/O curves can be modelled as being the result of two DPOAE components (Whitehead et al., 1992). One component is dominant at high stimulus levels. We will refer to this component as the high-level DPOAE. The other component is dominant for low stimulus levels, but saturates for higher stimulus levels. This component will be referred to as the low-level DPOAE.

Several experiments, performed in mammals (Kim, 1980; Mills, 1997), reptiles (Manley et al., 1993), birds

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Abbreviations: AP, amphibian papilla; BP, basilar papilla; DPOAE, distortion product otoacoustic emission; I/O curve, input–output curve; AP-DPOAE, distortion product otoacoustic emission from the amphibian papilla; BP-DPOAE, distortion product otoacoustic emission from the basilar papilla

(Froymovich et al., 1995), amphibia (Van Dijk et al., 2003) and insects (Coro and Kössl, 2001) have shown that low-level DPOAEs are more vulnerable to inner ear insult or death than high-level DPOAEs. This suggests that DPOAE are not only the composite of two components, but that these components are generated by two different mechanisms as well. In mammals, the low-level DPOAE is associated with the nonlinear outer hair cell mechanics, while the high-level DPOAE could be due to the passive mechanical properties of the basilar membrane (Mills et al., 1993). This idea of two emission components, arising from different mechanisms in the cochlea has been successfully incorporated in a model by Mills (1997). In this model, the low-level DPOAE arises around the peaks of the traveling waves of the two stimulus tones (his “peak” component), while the high-level DPOAE originates more basally in the cochlea (his “basal” component). Other models (e.g. Talmadge et al., 1998) that also incorporate two distinct emission components are only valid for low stimulus levels, and therefore not applicable for simulating DPOAE I/O curves. These models are able to predict other phenomena observed in DPOAE data for low stimulus levels (e.g. DPOAE fine-structure).

More recently, it has been shown that the presence of notches in I/O curves, and the differential vulnerability of high- and low-level DPOAEs may arise from a single nonlinearity (Lukashkin and Russell, 1998, 1999). The model calculations in these papers show that a Boltzman function, which is used to describe hair cell transduction, is sufficient to produce notches in amplitude data. In order to simulate the differential vulnerability of high- and low-level DPOAEs, the incorporation of a positive feedback mechanism in this model is necessary. Thus, both the two-source (i.e. Mills, 1997) and the one-source (i.e. Lukashkin and Russell, 1998, 1999) descriptions were successfully applied to describe DPOAE from the mammalian cochlea.

The anatomy of the frog’s inner ear differs considerably from the mammalian ear, the most prominent difference being the absence of a cochlea in amphibians. Instead, the inner ear of a frog holds two distinct papillae that are sensitive to airborne sound. The amphibian papilla (AP) consists of an S-shaped strip of hair cells, covered by a tectorial membrane (Frishkopf and Flock, 1974). It exhibits a tonotopic organization (Lewis et al., 1982), and the presence of traveling waves in the tectorial membrane has been proposed (Hillery and Narins, 1984). In *Rana pipiens pipiens*, afferent neurons from the AP are tuned to frequencies in the range of 0.1–1.25 kHz (Ronken, 1991). The basilar papilla (BP) is a much simpler structure. It consists of a small patch of sensory epithelium, partly covered by a tectorial membrane. The BP is of special interest to hearing research because it functions as a single auditory filter: almost all nerve fiber units are tuned to the same frequency

(Ronken, 1991), there is no evidence for traveling waves, and in ranid frogs the polarity of stereovillar bundles is identical for all hair cells (Lewis, 1978). In neither of these papillae is the sensory epithelium over a basilar membrane. Rather, hair cells are directly on a rigid support structure.

In the present study amplitude and phase of DPOAEs recorded from both the AP and the BP are presented. We will discuss to what extent our results are similar to those in other species, and whether mammalian models (Mills, 1997; Lukashkin and Russell, 1998, 1999) are applicable to the amphibian ear.

2. Materials and methods

The results presented were obtained from recordings in the right ear of five leopard frogs, *R. pipiens pipiens*. Across animals, body weight ranged from 35.5 to 58.6 g (average 46.4 g), and snout-vent length ranged from 7.4 to 8.4 cm (average 8.1 cm). In order to immobilize the animals, they were anaesthetized via an intramuscular injection of pentobarbital (Nembutal: 30 mg/kg) in one of the hindlimbs. During the experiment, the frog was covered by gauze, soaked in tap water, to prevent dehydration. Experiments were performed in a sound-attenuation room. All procedures were approved by the animal experiment committee of the Maastricht University.

A custom-built emission recording probe was placed against the skin surrounding the frog’s tympanic membrane. To reduce acoustic energy loss, silicone grease was used to obtain a tight seal between the probe and the skin. The probe contained two miniature speakers (E-A-RTONE 3A, Aearo Company, Indianapolis, IN, USA) for stimulus delivery, and a 1 in. high-sensitivity condenser microphone (B&K type 4179, Brüel & Kjær, Denmark) for emission recording. Stimulus tones, with frequencies f_1 and f_2 ($f_2 > f_1$) were generated from two separate DA channels (RP2, Tucker–Davis Technologies, Gainesville, FL, USA). The levels of the two stimulus tones (L_1 and L_2 , respectively) were controlled by separate programmable attenuators (PA5, TDT, Gainesville, FL, USA) and played through the miniature speakers.

The microphone signal was passed through a low-noise amplifier (B&K type 2660, Brüel & Kjær, Denmark; amplification = 20 dB) and a measuring amplifier (B&K type 2610, Brüel & Kjær, Denmark; amplification ≥ 10 dB). The amplified signal was recorded via an AD channel (RP2, TDT, Gainesville, FL, USA). The digitized microphone signal was stored on computer disc to be available for offline analysis. In order to monitor DPOAEs during the experiment the frequency spectrum of the amplified microphone signal was viewed online with a spectrum analyzer (SR760, Stanford Research

Systems, Sunnyvale, CA, USA). Customized software, developed in MATLAB (The MathWorks, Inc., Natick, MA, USA) and RPvds (TDT, Gainesville, FL, USA) was used to control stimulus tone generation and signal recording.

To determine the nonlinear distortion of the microphone and other recording equipment, measurements were conducted with the open end of the probe pressed against a hard surface. Across frequencies system distortion was about 90 dB below the primaries. Only for the lowest stimulus levels used ($L_1 = L_2 < 55$ dB SPL) did the system distortion disappear into the estimated noise floor. All DPOAEs were corrected for system distortion by vector-subtracting system distortion (as determined against the hard surface) from the stored microphone signals.

Primary frequency f_1 varied between 413 and 3212 Hz in ≈ 100 Hz steps, while the frequency ratio f_2/f_1 was kept constant at 1.1. For each f_1 , the stimulus level was decreased in 1 dB steps from 90 dB SPL down to stimulus levels for which DPOAEs were indistinguishable from the noise floor ($L_1 = L_2 = 51 - 41$ dB SPL). Only equal primary levels were used. The recording system was calibrated in situ using white noise at the start of the experiment.

For each parameter combination (f_1, L_1, f_2, L_2) the stimulus tones were played continuously for 50,000 sample points (≈ 4.10 s, with a sample frequency of 12.2 kHz). The frequency f_1 was chosen so that an integer number of sample points contained exactly 100 periods of this stimulus tone. Because the frequency ratio f_2/f_1 was constant at 1.1, not only f_1 , but also all other frequencies of interest were exactly periodic over the same number of sample points (i.e. 110 periods for f_2 , 90 periods for $2f_1 - f_2$, etc.). Depending on stimulus frequency f_1 , the 50,000 sample points contained a variable number of blocks in which f_1 had 100 periods (e.g. 16 blocks for $f_1 = 413$ Hz and 131 blocks for $f_1 = 3213$ Hz.)

The recorded microphone signal can be segmented in a fashion similar to the stimulus. From the recorded 50,000 sample points, the first periodic block and the trailing sample points, which did not make up an entire periodic block, were omitted from the analysis. The amplitudes (L_1, L_2) and phases (ϕ_1, ϕ_2) of the two stimulus tones were calculated using Fourier analysis. The parameters thus obtained were used to subtract the stimulus tones from the microphone signal in the time domain, leaving a signal containing only the DPOAEs and system noise. The periodic blocks in the remaining signal were subjected to a level-crossing artifact rejection method. Artifact free blocks were sub-averaged in two buffers A and B. The average $(A + B)/2$ can be used to estimate the DPOAEs amplitudes and phases, while the difference $A - B$ provides an estimate for the noise levels. The amplitude and phase of DPOAEs were calculated using Fourier analysis.

In these experiments, the phase of the stimulus tones was not controlled. To correct for phase variations of the stimulus tones across recordings, phase $\Phi_{nf_1-mf_2}$ of the DPOAEs will be given relative to the calculated phases of the stimulus tones:

$$\Phi_{nf_1-mf_2} = \phi_{nf_1-mf_2} - (n * \phi_1 - m * \phi_2),$$

where $\phi_{nf_1-mf_2}$ is the recorded DPOAE phase, and ϕ_1 , and ϕ_2 are the estimated phases of the primaries.

3. Results

3.1. DPOAE-audiograms

Distortion product otoacoustic emissions were detectable in all frogs investigated. An example of the DPOAE spectrum ($f_1 = 1822$ Hz; $L_1 = L_2 = 85$ dB SPL) is displayed in Fig. 1. Besides the large peaks corresponding to the stimulus tones, several distortion products are visible. In the present study the recorded data were systematically analyzed for distortion products at frequencies $2f_1 - f_2$, $3f_1 - 2f_2$, $2f_2 - f_1$ and $3f_2 - 2f_1$ (emboldened in Fig. 1). Fig. 2 shows DPOAE-audiograms of $2f_1 - f_2$, $3f_1 - 2f_2$, $2f_2 - f_1$ and $3f_2 - 2f_1$ DPOAEs for one animal. In general, amplitudes for DPOAE at $2f_1 - f_2$ and $2f_2 - f_1$ were larger than for $3f_1 - 2f_2$ and $3f_2 - 2f_1$.

DPOAE-audiograms showed a bimodal dependence of DPOAE level on frequency. Emission amplitudes were relatively small near $f_{dp} = 1250$ Hz. The DP-

Fig. 1. Spectrum of the averaged microphone signal, showing several DPOAE peaks from the basilar papilla of *R. pipiens pipiens*; $f_1 = 1822$ Hz, $f_2/f_1 = 1.1$, $L_1 = L_2 = 85$ dB SPL. The spectral peaks corresponding to the stimulus tones exceed the vertical scale (arrows). The combination tones are labelled by their frequency. For these high-level stimulus tones DPOAEs were observed at numerous frequencies. In the present study the recorded data were systematically analyzed for DPOAEs at $2f_1 - f_2$, $3f_1 - 2f_2$, $2f_2 - f_1$ and $3f_2 - 2f_1$. These DPOAEs are indicated in the figure by the emboldened labels and spectral peaks.

Fig. 2. Levels of distortion products at frequencies $2f_1 - f_2$ ('+', solid line), $3f_1 - 2f_2$ ('x', dashed line), $2f_2 - f_1$ ('o', solid line), $3f_2 - 2f_1$ ('Δ', dashed line) as a function of distortion product frequency. The DPOAE audiograms were recorded with $L_1 = L_2 = 70$ dB SPL, and $f_2/f_1 = 1.1$. Noise floor is approximately -33 dB SPL.

audiogram was similar in all animals studied. Based on characteristic frequencies of auditory nerve fibers from the AP and the BP (Ronken, 1991), we will refer to emissions with a frequency below 1250 Hz as AP-DPOAE (amphibian papilla DPOAE), and with frequencies above 1250 Hz as BP-DPOAE (basilar papilla DPOAE).

3.2. Amplitude and phase of $2f_1 - f_2$

In Fig. 3(a), I/O curves for $f_{dp} = 2f_1 - f_2$ are plotted in a three-dimensional format. In this figure, and all subsequent figures, only data points exceeding the estimated noise floor by 12 dB are shown ($12 \text{ dB} \approx 2 \times \text{SD}$ of the noise floor). Generally, the amplitude of the DPOAEs increases with increasing stimulus levels. An exception to this is the I/O curve at $f_{dp} = 908$ Hz, which shows a notch in amplitude around stimulus levels of 69 dB SPL.

The data are redrawn in Fig. 3(b) in the form of a contour plot. This figure shows that the bimodal dependence of DPOAE level on frequency, as seen in Fig. 1 for $L_1 = L_2 = 70$ dB SPL, is not restricted to that particular stimulus level but is present for all stimulus levels used. For high and intermediate stimulus levels ($L_1 = L_2 \geq 65$ dB SPL) the contour lines exhibit an inflection around $f_{dp} = 1250$ Hz. For low stimulus levels ($L_1 = L_2 \leq 65$ dB SPL) there is a region around 1250 Hz where DPOAE level does not exceed the estimated noise floor, although for both lower and higher frequencies, DPOAE are still detectable.

Visible in Fig. 3, but more clearly illustrated in Fig. 4(a), is a conspicuous difference between AP-DPOAE and BP-DPOAE I/O curves. Based on their

Fig. 3. (a) I/O curves of DPOAEs at $2f_1 - f_2$ in a three-dimensional format. Stimulus frequency ratio was fixed at $f_2/f_1 = 1.1$, while stimulus levels $L_1 = L_2$ were varied in 1 dB steps. Only data points exceeding the estimated noise floor by at least 12 dB are shown. The emboldened I/O curves are shown again in Fig. 4(a). (b) Data from (a) redrawn in the form of a two-dimensional contour plot. Contour lines are plotted at 5 dB intervals. As a reference the 0 dB SPL contour line is emboldened.

slopes, AP-DPOAE I/O curves can be divided into two segments. For the AP-DPOAE I/O curves, the segments are separated by a kneepoint at 70 dB SPL stimulus levels. At levels above the kneepoint, the DPOAE amplitude grows steeply (>1 dB/dB) with stimulus amplitude. Below the kneepoint, the DPOAE amplitude decreases with a shallow slope (<1 dB/dB) into the noise floor. At some AP frequencies the two segments were separated by a notch, rather than a kneepoint (for example Fig. 3(a), I/O curve at $f_{dp} = 908$ Hz). In those cases the amplitude of the DPOAE below the notch initially increases with decreasing stimulus levels. The shallow and steep slopes for low and high stimulus levels, respectively, are characteristic of the AP.

In contrast, the BP-DPOAE amplitude grows steeply with stimulus level (>1 dB/dB) for both low- and high-level stimuli. The low- and high-level segments were separated by a kneepoint (at 77 dB SPL stimulus level in Fig. 4(a)).

Fig. 4(b) shows the relative phase of the DPOAE I/O curves in Fig. 4(a). Phase data of individual I/O curves were unwrapped by removing 2π phase jumps and the phase was arbitrarily set to zero at $L_1 = L_2 = 90$ dB SPL. A close relation between the amplitude and phase can be seen. For stimulus levels associated with the notch/kneepoint in an I/O curve the phase changed rapidly. In contrast, in the stimulus level ranges for which the DPOAE amplitude grew with a constant slope the phase varied relatively little.

A complete overview of the relative phase corresponding to the DPOAE I/O curves from Fig. 3 is given

Fig. 4. (a) Individual DPOAE I/O curves for one AP-DPOAE frequency ($f_{dp} = 999$ Hz; '+', dashed line) and one BP-DPOAE frequency ($f_{dp} = 1895$ Hz; 'O', solid line). (b) Corresponding relative phase of the DPOAE. The phase has been set to zero at $L_1 = L_2 = 90$ dB SPL. Curves correspond to the emboldened lines in Figs. 3(a) and 5.

Fig. 5. (a) Relative phase of the DPOAE I/O curves from Fig. 3 in a three-dimensional format. For each I/O curve the phase has been unwrapped by removing 2π phase jumps. The unwrapped phase has been set to zero at $L_1 = L_2 = 90$ dB SPL. The emboldened phase curves are shown again in Fig. 4(b).

in Fig. 5. For low stimulus levels, the phase increasingly lagged the primary tones with increasing frequency, both for AP-DPOAE and BP-DPOAE. In other words, with decreasing stimulus levels the DPOAE group delay, defined as $\tau = -(1/2\pi)d\phi/df_{dp}$ of phase versus frequency curves, increased.

The phase characteristics for individual DPOAE I/O curves are similar over the entire frequency range. For each DPOAE I/O curve two segments can be seen where the phase changed relatively little. The transition between these segments was characterized by a rapidly changing phase.

3.3. Amplitude and Phase of other DPOAEs

Figs. 6 and 7 give an overview of the amplitude and associated phase data at DPOAE frequencies other than $2f_1 - f_2$. Besides the ranges of the frequency axes, the format of these figures is the same as those in Figs. 3 and 5. As was also shown in Fig. 2, amplitudes at $2f_1 - f_2$ and $2f_2 - f_1$ were larger than at $3f_1 - 2f_2$ and $3f_2 - 2f_1$. However, several similarities existed across the distortion components: (1) All DPOAE I/O curves showed an increase in amplitude with increasing stimulus levels. (2) This growth was with a constant slope for low and high stimulus levels. For intermediate stimulus levels the slopes of DPOAE I/O curves decreased, resulting in either a notch or a kneepoint. (3) For intermediate stimulus levels, associated with the decreased slope of the I/O curve, the phase changed rapidly. Away from intermediate stimulus levels, this change in phase was more gradual or absent. (4) The

Fig. 6. Amplitude of several DPOAEs in the form of two-dimensional contour plots. Except for the frequency range, the format is the same as in Fig. 3(b). (a) $2f_2 - f_1$, (b) $3f_1 - 2f_2$ and (c) $3f_2 - 2f_1$. Amplitude contour lines are drawn at 5 dB intervals. For reference the 0 dB SPL contour line is emboldened.

group delay of DPOAE-audiograms increased with decreasing stimulus levels.

There were clear differences between emissions with $f_{dp} < f_1, f_2$, i.e. $2f_1 - f_2$ and $3f_1 - 2f_2$, and emissions with $f_{dp} > f_1, f_2$, i.e. $2f_2 - f_1$ and $3f_2 - 2f_1$. In the BP frequency region the tuning of individual contour lines (Figs. 3(b) and 6) was much wider for DPOAE at $2f_2 - f_1$ and $3f_2 - 2f_1$ compared to DPOAE at $2f_1 - f_2$

Fig. 7. Relative phase of the DPOAE I/O curves from Fig. 6 in a three-dimensional format. The displayed frequency range is different from that in Fig. 4. (a) $2f_2 - f_1$, (b) $3f_1 - 2f_2$ and (c) $3f_2 - 2f_1$. As in Fig. 5, the phase has been unwrapped, and the phase thus obtained has been set to zero at $L_1 = L_2 = 90$ dB SPL.

and $3f_1 - 2f_2$. For instance, the widths of the 0 dB SPL contour lines (emboldened black lines in Figs. 3(b) and 6) at 5 dB above the minimum were 452 and 440 Hz for $2f_1 - f_2$ (Fig. 3(b)) and $3f_1 - 2f_2$ (Fig. 6(b)), respectively. For $2f_2 - f_1$ (Fig. 6(a)) and $3f_2 - 2f_1$ (Fig. 6(c)) these widths were 731 and 1208 Hz, respectively. Besides this difference in tuning widths, a clear separation between AP-DPOAE and BP-DPOAE can be seen for $2f_1 - f_2$ (Fig. 3(b)) and $3f_1 - 2f_2$ (Fig. 6(b)). For DPOAE at $2f_2 - f_1$ (Fig. 6(a)) and $3f_2 - 2f_1$ (Fig. 6(c)) this separation is absent; DPOAE amplitude does not decrease when crossing from AP-DPOAE to BP-DPOAE.

Fig. 8. I/O curves for several DPOAEs from the basilar papilla, all evoked with the same set of stimulus tones; $f_1 = 1822$ Hz, $f_2/f_1 = 1.1$, $2f_1 - f_2 = 1640$ Hz ($\circ$, solid line); $2f_2 - f_1 = 2186$ Hz ($+$, dashed line); $3f_1 - 2f_2 = 1458$ Hz ($\triangle$, solid line); $3f_2 - 2f_1 = 2369$ Hz ($\times$, dashed line). Based on their slopes, I/O curves can be divided into two segments; one segment is evoked with low level stimulus tones and the other with high level stimulus tones. For the different DPOAEs, the transition from one segment to the other occurs at different levels of the stimulus tones: $2f_1 - f_2$: 69 dB SPL; $2f_2 - f_1$: 72 dB SPL; $3f_1 - 2f_2$: 74 dB SPL; $3f_2 - 2f_1$: 76 dB SPL.

A comparison between the various DPOAEs ($2f_1 - f_2$, $2f_2 - f_1$, $3f_1 - 2f_2$ and $3f_2 - 2f_1$) evoked with a particular set of stimulus frequencies, showed that for each DPOAE frequency the transition from the low-level to the high-level segment occurred at a different stimulus level. This is illustrated in Fig. 8 for one set of DPOAE I/O curves from the basilar papilla ($f_1 = 1822$ Hz). Here, each I/O curve exhibits a kneepoint or a notch at different stimulus levels: $2f_1 - f_2$: 69 dB SPL; $2f_2 - f_1$: 72 dB SPL; $3f_1 - 2f_2$: 74 dB SPL; $3f_2 - 2f_1$: 76 dB SPL. This can also be inferred from the various contour plots (Figs. 3(b) and 6). The transition from low-level to high-level DPOAE is marked by a decrease in the slope of the I/O curve, which is reflected in a larger distance between the contour lines in the contour plots. In Figs. 3(b) and 6 the contour lines are further apart around those stimulus levels for which the DPOAE amplitude is approximately 0 dB SPL (emboldened black lines). This emboldened black line occurs at different stimulus levels in the different contour plots, indicating that the transition from low-level to high-level DPOAE occurs at different stimulus levels for the different DPOAE.

3.4. Slopes of DPOAE I/O curves

Commonly, slopes of DPOAE I/O curves are determined by fitting straight lines to those parts of the I/O

curve for which the slope is approximately constant (e.g. Mills and Rubel, 1996; Coro and Kössl, 2001). This method requires the choice of a stimulus level range for which the DPOAE I/O curve is assumed to be linear. To avoid this subjective definition of stimulus ranges we calculated the slope of the DPOAE I/O curve in each data point X by fitting a straight line through its two neighboring points (i.e. the data points evoked with stimulus levels 1 dB above and below the levels of X itself).

Slopes, combined for all DPOAE frequencies and all frogs investigated are shown in Fig. 9 in histograms. Based on stimulus level, both AP-DPOAE and BP-DPOAE were subdivided in a low- and a high-level part. Fig. 9(a) highlights the difference in slope between low and high-level slopes for AP-DPOAE. High-level slopes averaged 1.96 dB/dB while low-level slopes averaged 0.44 dB/dB. For BP-DPOAE slopes (Fig. 9(b)) these

Fig. 9. Comparison of the growth rate of DPOAEs from the AP and the BP. DPOAE I/O curves were grouped as being either AP-DPOAE ($f_{dp} < 1250$ Hz) or BP-DPOAE ($f_{dp} > 1250$ Hz). Within each group individual DPOAE I/O curves were divided in two subgroups: stimulus levels > 70 dB SPL and stimulus levels < 65 dB SPL. (a) Distribution of observed slopes of high and low stimulus levels for the amphibian papilla. Average slopes: high stimulus levels: 1.96 dB/dB; low stimulus levels: 0.44 dB/dB. (b) Same as (a), but for the basilar papilla. Average slopes: high stimulus levels: 1.82 dB/dB; low stimulus levels: 1.30 dB/dB. Histograms were scaled to a peak value 1.

Fig. 10. Difference between the 60 and 90 dB SPL DPOAE phase as a function of distortion product frequency. (a) $2f_1 - f_2$, (b) $3f_1 - 2f_2$, (c) $2f_2 - f_1$ and (d) $3f_2 - 2f_1$. Each symbol type denotes data from one frog. The vertical dashed line gives the frequency separation between AP and BP (1250 Hz; Ronken, 1991).

averages were 1.82 and 1.30 dB/dB for high- and low-level slopes, respectively.

3.5. Phase difference between low- to high-level DPOAE

In order to quantify the phase change associated with the transition from low- to high-level DPOAE, Fig. 10 displays the phase difference between DPOAE evoked with stimulus levels of 60 and 90 dB SPL, respectively. Results for all frogs were combined, where different frogs have different symbols. Each distortion product is represented in a different panel. Both for AP-DPOAE and BP-DPOAE, and for all distortion products, the phase difference increases systematically with increasing frequency. For $2f_1 - f_2$, $3f_1 - 2f_2$ and $3f_2 - 2f_1$ the phase difference is up to 1.5π and for $2f_2 - f_1$ this difference is up to 2π .

4. Discussion

4.1. Bimodal shape of DPOAE-audiograms

All DPOAE-audiograms (directly recorded or reconstructed from DPOAE I/O curves) obtained in the frog showed a bimodal dependence on frequency. Two frequency regions were found where relative maxima in DPOAE amplitude occurred. The frequency ranges of these two regions coincided with the frequency ranges of the tuning of single nerve fibers from the two hearing papillae. This suggests that, for all stimulus levels investigated, DPOAEs arose in these papillae. The same

conclusion was drawn by Van Dijk and Manley (2001) and Van Dijk et al. (2002).

Interestingly, DPOAEs were not found for distortion product frequencies below approximately 500 Hz, although the AP is innervated by nerve fibers tuned to frequencies as low as 100 Hz (Frishkopf and Goldstein, 1963; Frishkopf et al., 1968; Feng et al., 1975). A similar observation was made for low frequency DPOAEs in the treefrog, *H. cinerea* (Van Dijk and Manley, 2001). The absence of low frequency DPOAEs might be related to the fact that the tuning of the low frequency (rostral) part of the AP in *R. pipiens pipiens* is based on electrical resonance of the hair cells (Smotherman and Narins, 1999). However, in the bobtail lizard (Manley et al., 1993) DPOAEs have been recorded in the frequency range which probably relies on electrical resonance as tuning mechanism (Manley, 2002). Thus, the absence of DPOAEs may not be a fundamental characteristic of electrical hair cell tuning.

Alternatively, it might be that DPOAEs are generated in the low frequency part of the AP, but that they are attenuated below detectable levels before being emitted. The tectorial membrane on the rostral portion of the AP is a rather massive structure, which may impede any low frequency DPOAEs generated by the underlying hair cells (Lewis et al., 1982).

4.2. Growth of DPOAE I/O curves

The data presented in this paper showed that the slopes of DPOAE I/O curves vary with stimulus tone level, similar to what has been found in mammals (e.g. Kim, 1980) and other vertebrates (e.g. Taschenberger and Manley, 1998). We will discuss three possible mechanisms to explain the decreased slopes of DPOAE I/O curves around intermediate stimulus levels in amphibians.

The first way to explain a transition from low- to high-level behavior is a change in vibrational mode of the structures involved in the inner ear. If the mode of vibration changes at some stimulus levels, the transition in DPOAE behavior is expected to occur at this transition stimulus level for all DPOAE orders. In contrast, our data show that the transition from low- to high-level DPOAE takes place at lower stimulus levels for $2f_1 - f_2$ and $2f_2 - f_1$ than for $3f_1 - 2f_2$ and $3f_2 - 2f_1$ (Fig. 8). This observation excludes a vibration-mode transition as the origin of notches/kneepoints in DPOAE I/O curves.

A second explanation for the presence of notches/kneepoints in DPOAE I/O curves involves the presence of a single DPOAE source. Lukashkin and Russell (1998, 1999) presented a model, based on the receptor potential nonlinearities of single hair cells. With their model they could account for the occurrence of notches and phase changes in DPOAE I/O curves. This model

seems applicable to the generation of DPOAE I/O curves especially from the frog's BP. Not only are all nerve fibers tuned to a single frequency in this papilla, the absence of traveling waves, and the unipolarity of all hair bundles effectively reduce the entire papilla to a "single hair cell". The model of Lukashkin and Russell always gives a change in phase of exactly π rad across the kneepoint/notch in amplitude data. The phase data presented here, both for AP-DPOAE and BP-DPOAE showed phase shifts between 0 (no phase shift) and 2π rad. This result excludes this type of model to be applicable to DPOAE generation in the amphibian inner ear.

The third possible explanation involves the interference between two discrete DPOAE components (Whitehead et al., 1992; Manley et al., 1993). One component is assumed to be dominant for high stimulus levels. The other component dominates for low stimulus levels, but saturates for higher stimulus levels. The amplitude and phase curves from the amphibian ear all show a transition from low- to high-level stimuli. Consequently, our data are consistent with a description of DPOAEs originating from two distinct sources, as has been proposed for mammals.

A recent model used to simulate DPOAE I/O curves from the cochlea postulates that the two DPOAE sources are at different tonotopic locations in the cochlea (Mills, 1997). The low-level component arises around the place where the stimulus tones result in maximum deflection of the basilar membrane, while the high-level component originates more basally in the cochlea. This model can successfully predict several features of DPOAE I/O curves recorded from small mammals. Since the AP exhibits a tonotopic organization, a similar model may be applicable to the AP, although a traveling wave has not yet been identified. In contrast, the BP lacks a tonotopic organization and essentially functions as a single auditory filter (Ronken,

1991). Nevertheless, it may be that a two-source model is applicable to the BP, with each source in a different functional/anatomical structure of the papilla.

4.3. Slopes of DPOAE I/O curves

For high-level stimulus tones, DPOAE I/O curves from both papillae in the frog show similar growth characteristics. On the other hand, for low-level stimulus tones there was a conspicuous difference between the slopes of DPOAE I/O curves from the AP and the BP. We will separately discuss the properties of AP- and BP-DPOAE I/O curves, starting with the AP. Parallel with this, comparisons with DPOAE I/O curves recorded in various other vertebrates will be made.

The slope of DPOAE I/O curves in mammals, birds and reptiles depends on the level of the stimulus tones. For low stimulus levels the growth is usually shallow, 1 dB/dB or less (see Table 1 for an overview and references). In some species the slope shows a dependency on frequency (humans; Lonsbury-Martin et al., 1990; birds; Kettembeil et al., 1995), but for example in rabbits (Whitehead et al., 1992), this trend is absent. In contrast, for higher stimulus levels DPOAE growth is steep, and usually much larger than 1 dB/dB. Our results show that DPOAE growth in the frog's amphibian papilla is similar to that in mammals, birds and one reptile.

As discussed above, a possible explanation for the presence of kneepoints/notches in DPOAE I/O curves is the involvement of two generation mechanisms. As we will argue, one may be passive, the other active. Here, an active mechanism refers to a system for which the signal energy in the output does not originate from the input signal alone. For example, in the vertebrate hair cell, the transduction current is controlled by transduction channel opening, but a substantial part of the energy derives from the electrical potential gradient across the channel. Thus, forward transduction is evidently active.

Table 1

Slopes of DPOAE I/O curves as reported in literature for several species from the phylum *Chordata* (all classes except *Pisces*)

	≤ 1 dB/dB	> 1 dB/dB
High level slope	None	All species investigated, i.e. Frog AP + BP [1, this work] Alligator lizard [2] Bobtail lizard [3] Chicken [4–6] Starling [5] Mammals [7–16]
Low level slope	Frog AP [1, this work] Bobtail lizard [3] Chicken [4–6] Starling [5] Mammals [7–16]	Frog BP [1, this work] Alligator lizard [2]

The slopes reported are for the distortion product at $2f_1 - f_2$ and for DPOAE I/O curves where both primary tone levels were simultaneously varied. Due to the use of various ways of determining the slopes, as well as the various amount of detail (e.g. frequency dependency) in the different references, we simplified by only reporting the slopes with reference to a slope of 1 dB/dB. For a slope < 1 dB/dB, the underlying nonlinear system is compressive. A slope > 1 dB/dB corresponds to an expansive nonlinearity. Not all references explicitly state the slopes of DPOAE I/O curves. In those cases we estimated the slope from the figures presented. References: [1] Van Dijk et al. (2003); [2] Rosowski et al. (1984); [3] Manley et al. (1993); [4] Froymovich et al. (1995); [5] Kettembeil et al. (1995); [6] Sun et al. (2000); [7] Abdala (2000); [8] Brown (1987); [9] Dorn et al. (2001); [10] Dreisbach and Siegel (2001); [11] Gaskill and Brown (1990); [12] Kim (1980); [13] Lonsbury-Martin et al. (1987); [14] Lonsbury-Martin et al. (1990); [15] Mills and Rubel (1996); [16] Whitehead et al. (1992).

For a biological system, active processes rely on metabolism. Disruption of the healthy physiological condition of the system will deteriorate active mechanisms. Across species, it has been shown that the low-level mechanism is more vulnerable to acoustic trauma, anoxia and pharmacological agents than the mechanism dominant at high stimulus levels (mammals: Zurek et al., 1982; reptiles: Manley et al., 1993; birds: Sun et al., 2000; amphibia: Van Dijk et al., 2003). This differential vulnerability has been interpreted as being evidence for the involvement of an active mechanism in the generation of low-level DPOAE, while the mechanism involved in the generation of high-level DPOAE may originate from the passive nonlinear response of the inner ear.

In mammals, somatic outer hair motility (Brownell et al., 1985) has been proposed to be this active mechanism. It has been shown that this somatic motility relies on the presence of the protein prestin in the hair cell basolateral membrane (Zheng et al., 2000), and its effect on DPOAE thresholds shows that it is a critically important component of the mammalian cochlear amplifier (Liberman et al., 2002). In amphibians, and other nonmammalian vertebrates, there is no evidence of somatic motility of the hair cells (He et al., 2003; Manley, 2001).

An alternative amplifier may be the active movement of hair bundles. This movement has been shown *in vitro* in both nonmammalian vestibular systems (Hudspeth, 1997) and the hearing organ of turtles (Crawford and Fettiplace, 1985). *In vivo* evidence for active movement of hair bundles has been presented for the bobtail lizard (Manley, 2002). Possibly, active hair bundle movement also occurs in mammalian outer hair cells (Kennedy et al., 2003). Hence, it is conceivable that active hair bundle movement might be an active mechanism in mammals and nonmammals. In mammals this may act in parallel with somatic motility of outer hair cells.

In the BP the properties of DPOAE I/O curves were markedly different from those in the AP (Fig. 9). The slopes of BP-DPOAE I/O curves evoked with both low and high stimulus levels was greater than 1 dB/dB. This is at variance from all other vertebrates investigated except the alligator lizard (component 1 and 2 in Rosowski et al., 1984; Table 1). Both in the frog BP and in the alligator lizard, the slopes of the growth functions are similar for low and high stimulus levels.

Two observations suggest that the frog basilar papilla may be a passive auditory receiver: (1) DPOAEs from the BP are less sensitive to physiological trauma than DPOAEs from the AP (Van Dijk et al., 2003) and (2) the apparent absence of spontaneous otoacoustic emissions from the BP (Van Dijk and Manley, 2001). Possibly, the steep growth of DPOAEs, as found in the AP at high stimulus levels and in the BP at both low and high stimulus levels, is a signature of passive DPOAE gen-

eration. But how can BP-DPOAE be passively generated, if the transduction mechanism is active? Possibly, BP-DPOAE arise from the nonlinear stiffness of hair bundles. Although this nonlinear stiffness involves the transduction channel (Howard and Hudspeth, 1988), it does not rely on the active forward transduction current. Thus, DPOAEs from the BP may passively be generated.

On the other hand, in the alligator lizard spontaneous otoacoustic emissions have been found (Manley, 2000) over the same frequency range for which the DPOAE I/O curves show steep slopes at both low and high stimulus levels. Thus, although the frog BP and the alligator lizard take a special position with respect to their DPOAE properties, the implications of this are not yet clear.

The phase change associated with the transition from low to high stimulus levels systematically increased with increasing frequency (Fig. 10). This suggests that at low stimulus levels the effective dynamic order of the basilar papilla is larger than for high stimulus levels. Possibly, with the saturation of the low-level DPOAE at intermediate stimulus levels, some dynamic orders saturate, and the BP effectively becomes a lower-order system.

4.4. Conclusion

In the present paper we have shown that, based on their slopes, DPOAE I/O curves from the amphibian ear can be divided into two segments: one for low stimulus levels and one for high stimulus levels. The transition between these two segments in the amplitude data are accompanied by a phase change. A model based on the receptor potential nonlinearities of single hair cells (Lukashkin and Russell, 1998) cannot explain the observed emission behavior. Also, the shape of the DPOAE I/O curves cannot be accounted for by assuming a transition in the mode of vibration of the inner ear structures. Rather, our findings are in agreement with the hypothesis that DPOAE I/O curves are the composite of two components, each of which is dominant for a different range of stimulus levels.

For low-level stimulus tones, the slopes of I/O curves showed a conspicuous difference between AP-DPOAE and BP-DPOAE. This difference in slopes suggest important differences between DPOAE mechanisms in the AP and the BP. Based on the observations that the generation of spontaneous otoacoustic emissions is limited to the AP (Van Dijk and Manley, 2001) and that low-level AP-DPOAE are more vulnerable to inner ear insults than low-level BP-DPOAE (Van Dijk et al., 2003) we hypothesize that in the amphibian papilla the generation of the low-level DPOAE component involves an active mechanism. In contrast, for the basilar papilla we suggest that DPOAEs originate from passive nonlinearities.

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