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Meenderink, SWF; van Dijk, P

Published in:
Journal of the Acoustical Society of America

DOI:
[10.1121/1.1925887](https://doi.org/10.1121/1.1925887)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Meenderink, SWF., & van Dijk, P. (2005). Characteristics of distortion product otoacoustic emissions in the frog from L-1,L-2 maps. *Journal of the Acoustical Society of America*, 118(1), 279-286.
<https://doi.org/10.1121/1.1925887>

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Characteristics of distortion product otoacoustic emissions in the frog from L_1, L_2 maps

Sebastiaan W. F. Meenderink

Department of Otorhinolaryngology and Head & Neck Surgery, University Hospital Maastricht,
P.O. Box 5800, 6202 AZ Maastricht, and Institute for Brain and Behaviour, Maastricht University,
The Netherlands

Pim van Dijk

Department of Otorhinolaryngology, University Medical Centre Groningen, P.O. Box 30.001, 9700 RB
Groningen and School of Behavioral, and Cognitive Neurosciences, University of Groningen, The Netherlands

(Received 22 November 2004; revised 8 April 2005; accepted 11 April 2005)

For a given set of stimulus frequencies (f_1, f_2), the level of distortion product otoacoustic emissions (DPOAEs) varies with the levels of the stimulus tones. By variation of the stimulus levels, L_1, L_2 -maps for DPOAEs can be constructed. Here, we report on L_1, L_2 -maps for DPOAEs from the frog ear. In general, these maps were similar to those obtained from the mammalian cochlea. We found a conspicuous difference between the equal-level contour lines for low-level and high-level DPOAEs, which could be modeled by a saturating and an expansive nonlinearity, respectively. The transition from the high-level to the low-level response was accompanied by a DPOAE phase-change, which increased from 0 to π rad with increasing frequency. These results suggest that in the frog low-level and high-level DPOAEs are generated by separate nonlinear mechanisms. Also, there was a conspicuous difference in the growth of the low-level emissions from the two anuran auditory papillae. In the basilar papilla, this growth was expansive for the lowest stimulus levels and saturated for intermediate levels. This is consistent with the behavior of a Boltzman nonlinearity. In the amphibian papilla this growth was compressive, suggesting the additional effect of a compressive amplification mechanism on the generation of DPOAEs. © 2005 Acoustical Society of America. [DOI: 10.1121/1.1925887]

PACS number(s): 43.64.Bt, 43.64.Jb, 43.80.Lb [BLM]

Pages: 279–286

I. INTRODUCTION

Otoacoustic emissions (OAEs) are weak sounds that can be measured in the ear canal, both with and without the presence of an external stimulus. When the stimulus consists of two pure tones, with appropriately chosen frequencies (f_1, f_2) and amplitudes (L_1, L_2), so-called distortion product OAEs (DPOAEs) can be recorded at various intermodulation frequencies of the two stimulus tones (e.g., $2f_1 - f_2$ and $2f_2 - f_1$). To date, most studies of DPOAEs have been on mammals, for which it is generally assumed that (at least for low-level stimuli) electromotility of the outer hair cells (Brownell *et al.*, 1985) is the responsible mechanism for OAE generation. As such, DPOAEs provide a noninvasive tool to investigate the (nonlinear) mechanics of hair cells that are involved in the transduction of sound.

When, for a given combination of stimulus frequencies, the levels of the two stimulus tones are varied, the input/output (I/O) characteristics of DPOAEs may be determined. In such experiments typically both stimulus levels are varied, while keeping the difference $L_1 - L_2$ fixed. The resulting I/O curves exhibit a complex shape, with the rate of DPOAE-amplitude growth dependent on absolute stimulus levels (mammals: Brown, 1987; birds: Froymovich *et al.*, 1995; reptiles: Manley *et al.*, 1993; amphibians: Meenderink and Van Dijk, 2004). One way to explain the shape of these I/O curves (and associated DPOAE phase) is by the interference of two level-dependent components, of which each is domi-

nant over a different range of stimulus levels (Whitehead *et al.*, 1992). The different vulnerability of these components has led to the suggestion that they arise from different mechanisms. The low-level component presumably arises from “active” (i.e., energy is supplied by the ear in such a manner that sound-induced vibration is enhanced) hair cell mechanics, while the component that dominates for high-level stimuli is generated by some “passive” (i.e., no energy is supplied by the ear) nonlinearity, that is not necessarily associated with hair cells (Mills *et al.*, 1993).

Recently, doubt has risen as to whether such a two-source model is necessary to account for DPOAE generation in the mammalian cochlea (Mills, 2002). It seems that the division into a passive high-level and an active low-level component is no longer supported (Mom *et al.*, 2001; Frolenkov *et al.*, 1998). The results from these and other studies in mammals indicate that the generation of low-level and high-level DPOAEs both involve an active nonlinear mechanism that critically depends on the normal functioning of the outer hair cells. In confirmation with this, several I/O characteristics (i.e., differential vulnerability and notches) can be accounted for by models that incorporate only a single, active mechanism for DPOAE generation (Mills, 1997; Lukashkin *et al.*, 2002). Seemingly, only one type of nonlinear mechanism is necessary for DPOAE generation. However, mice lacking the protein prestin in their hair cell’s basolateral membrane do generate high-level DPOAEs

(Liberman *et al.*, 2004). Apparently, in contrast to low-level DPOAEs in mice, high-level DPOAEs do not depend on prestin motility and thus must originate from another mechanism.

In frogs, the anatomy of the inner ear deviates considerably from that found in mammals, the most prominent difference being the absence of a cochlea. Instead, the frog inner ear contains two papillae that respond to airborne sound. Of these, the amphibian papilla (AP) shows several similarities with the cochlea. It consists of an elongated strip of sensory epithelium that exhibits tonotopic organization (Lewis *et al.*, 1982). As in the cochlea, the entire epithelium is covered by a tectorial membrane. Hair cells are innervated by both efferent and afferent nerve fibers, the latter having tuning characteristics similar to those found in mammals (Lewis, 1992). The AP may generate both spontaneous and distortion product OAEs (Van Dijk and Manley, 2001). Different from the cochlea are the absence of a basilar membrane and of distinct groups of hair cells (i.e., no inner and outer hair cells).

The structure of the other papilla, the basilar papilla (BP), is much simpler. It consists of a small patch of sensory epithelium, covered by a tectorial membrane. In *R. catesbeiana*, the entire papilla contains between 50 and 100 hair cells whose stereovillar bundles are oriented identically (Lewis, 1978). As in the AP, this papilla lacks a basilar membrane and hair cells are not differentiated into differing groups. The BP lacks tonotopic organization; in individual frogs, the majority of afferent nerve fibers is tuned to a single frequency (Ronken, 1991; Van Dijk *et al.*, 1997). These properties ensure that the entire papilla essentially functions as a single auditory filter (Ronken, 1990; Van Dijk and Manley, 2001). The BP may generate distortion product OAEs, but no spontaneous OAEs have been reported from this papilla.

Recently, we presented I/O curves for DPOAEs from the frog ear that are very similar to I/O curves obtained in other vertebrates (Meenderink and Van Dijk, 2004). We suggested that DPOAEs in the frog are the result from two level-dependent components, with each component arising from a different nonlinear mechanism. Here, we further detail the stimulus-level dependence of DPOAEs in the frog by presenting full L_1, L_2 maps. These maps showed a differential dependence of high-level and low-level DPOAEs, as is evidenced by the different contour lines. Combined with the observed frequency-dependent phase change associated with the transition from low-level to high-level DPOAEs, these results provide further support for the involvement of two separate, level-dependent emission-generation mechanisms in the frog.

II. MATERIALS

Distortion product otoacoustic emissions were recorded from 12 ears of 7 Northern leopard frogs, *Rana pipiens pipiens*; body mass; 37.6–46.8 g (mean: 42.7 g). In order to immobilize the frog during DPOAE recording, it was anaesthetized with an intramuscular injection of pentobarbital sodium solution (diluted Nembutal: 6.0 mg/ml; 10 μ l/g body weight) in one of the hind limbs. Approximately 25 min after

the administration of the anaesthetic agent, the frog was transferred to a sound-attenuating chamber and placed between wet gauze to prevent dehydration and to facilitate cutaneous respiration.

A custom-built probe assembly was carefully placed with its open end against the skin surrounding the tympanic membrane. A tight seal between the probe and the skin was obtained using vaseline. The probe assembly contained two miniature speakers (E-A-RTONE 3A, Aero Company, IN) for stimulus delivery, and a 1 in. high-sensitivity condenser microphone (B&K type 4179, Brüel & Kjær, Denmark) for emission recording. DPOAEs were evoked by stimulating the ear with two simultaneously played pure tones. These stimulus tones, with frequencies f_1 and f_2 ($f_2 > f_1$), were generated from two separate D/A channels (RP2, Tucker-Davis Technologies, Gainesville, FL), attenuated in order to set their levels (PA5, TDT, Gainesville, FL), and each one was played through a separate miniature speaker.

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 ≥ 0 dB). This amplified microphone signal was recorded using an A/D channel (RP2, TDT, Gainesville, FL) and stored on computer disk for offline analysis. In order to monitor the DPOAEs during the experiment, the frequency spectrum of the amplified microphone signal was viewed online using a spectrum analyzer (SR760, Stanford Research Systems, Sunnyvale, CA). Customized software, written in MATLAB (The Mathworks Inc., Natick, MA) and Rpvds (TDT, Gainesville, FL) was used to control stimulus tone generation and signal recording. The recorded signal was averaged in the time domain after the application of a level-crossing artifact-rejection method. From the averaged signal, both the DPOAEs and the noise floor were calculated using Fourier analysis. For further details concerning data acquisition and analysis we refer to Meenderink and Van Dijk (2004).

Data were collected at various different stimulus frequency combinations (overall range: $313 < f_1 < 3213$ Hz; $f_2/f_1 = 1.02, 1.1, \text{ or } 1.3$). For each f_1, f_2 combination, stimulus levels were independently and randomly varied over a 30 dB range in 3 dB steps. This resulted in a square L_1, L_2 matrix, containing 121 points for each stimulus frequency combination.

To determine the distortion of the recording system, additional experiments were performed with the open end of the probe assembly closed by a solid surface. In general, system distortion at the frequencies of the cubic difference tones was approximately 90 dB below the stimulus levels. Only for low stimulus level combinations did this system distortion disappear in the noise floor. Based on these recordings, only DPOAEs for which the amplitude exceeded both the noise floor and the system distortion were arranged in L_1, L_2 matrices. This amounts to the presentation of DPOAEs whose amplitudes exceeded -15 dB SPL. Since the phase of the two stimulus tones was not controlled in the experiments, the DPOAE phase will be given relative to the phase of the

two stimulus tones according to $\Phi_{nf_1-mf_2} = \phi_{nf_1-mf_2} - (n * \phi_1 - m * \phi_2)$, where $\phi_{nf_1-mf_2}$ is the recorded DPOAE phase, and ϕ_1 and ϕ_2 are the calculated phases of the two stimulus tones.

All procedures were approved by the animal experiment committee of the Maastricht University.

III. RESULTS

Distortion product otoacoustic emissions (DPOAEs) could be detected in all ears investigated. Although for certain stimulus tone combinations, DPOAEs could be observed at up to 8 different distortion frequencies, here only DPOAEs at frequencies $2f_1-f_2$ and $2f_2-f_1$ will be presented. The DPOAE data were visualized by plotting both the amplitude and the phase of the DPOAE against the levels of both stimulus tones. Within such an L_1, L_2 map, contour lines of equal emission amplitude were calculated at 3 dB intervals. Only data points with amplitudes > -15 dB SPL were included. Phase was represented by arrows, with the phase for the largest L_1, L_2 combination arbitrarily set to zero. As such, absolute phase holds no meaning, but phase changes within each L_1, L_2 map do.

Figure 1 displays results for DPOAEs at $2f_1-f_2$ [Fig. 1(a)] and $2f_2-f_1$ [Fig. 1(b)] obtained from the frog basilar papilla ($f_1=1913$ Hz, $f_2/f_1=1.02$ with L_1 and L_2 between 56 and 86 dB SPL). In this figure, the 3 dB intervals between consecutive amplitude contour lines are coded in gray, with the corresponding DPOAE amplitudes given in the key. Focusing on the amplitude, each L_1, L_2 map could be divided into two principal regions based on the shape of the contour lines. For relatively low stimulus levels, the contour lines were clearly convex. As a result, these lines encompass a more or less pronounced ridge in the L_1, L_2 map that lies approximately parallel to the diagonal $L_1=L_2$. In contrast, when one or both of the stimulus levels were relatively high, the contour lines approximated straight lines, with consecutive contour lines being parallel to each other. The slope of these contour lines depended on the DPOAE frequency: $L_2/L_1=-2$ dB/dB for $2f_1-f_2$ and $L_2/L_1=-0.5$ dB/dB for $2f_2-f_1$.

For the DPOAE phase, the L_1, L_2 maps could be divided into the same two principal regions as the amplitude data (i.e., two relatively low-level stimuli versus at least one relatively high-level stimulus). Within each of these two regions the phase was relatively constant, while between them the phase was different. In the examples shown, this phase difference is approximately 0.7π rad for $2f_1-f_2$ [Fig. 1(a)] and 0.9π rad for $2f_2-f_1$ [Fig. 1(b)]. For clarity, we will refer to the two principal regions in the L_1, L_2 maps as “low level” (relatively low stimulus levels) and “high level” (at least one of the two stimulus levels is relatively high), respectively.

In frogs, DPOAEs arise from either one of the two hearing papillae, depending on the stimulus frequencies used (Van Dijk and Manley, 2001; Meenderink and Van Dijk, 2004). This is reflected in the bimodal shape of the DPOAE audiograms, as illustrated in Figs. 2(a) and 2(b). DPOAEs evoked with low-frequency stimulus tones ($f_1 < 1250$ Hz for $2f_1-f_2$ and $f_1 < 1000$ Hz for $2f_2-f_1$, respectively) arise from

FIG. 1. Amplitude and phase of DPOAEs at (a) $2f_1-f_2$ and (b) $2f_2-f_1$ as function of stimulus levels L_1 and L_2 . Shown are data recorded in one frog for $f_1=1913$ Hz and $f_2/f_1=1.02$. This combination of stimulus frequencies results in DPOAEs originating from the frog basilar papilla. The DPOAE amplitude is represented by the equal-amplitude contour lines. The lowest contour lines are at -15 dB SPL and subsequent contour lines are drawn in 3 dB increments. The consecutive intervals between the contour lines are coded in gray, as given in the key. The phase of the DPOAEs is given by the arrows overlaying the contour lines, with a full counterclockwise rotation of the arrows corresponding to a phase lag of 2π rad. The phase is relative to the phase at $L_1=L_2=86$ dB SPL.

the amphibian papilla, while DPOAEs evoked with larger stimulus frequencies originate from the frog basilar papilla. This strict separation between DPOAE generation from the AP and the BP is probably not true. Stimulus frequencies around 1250 Hz presumably evoke an emission component in each papilla. These components will combine, which may result in the amplitude trough commonly seen in DPOAE audiograms.

As an overview, the remaining subplots in Fig. 2 show L_1, L_2 maps at 4 stimulus frequency combinations, all obtained in one ear. For reference, the stimulus frequencies f_1 for which these L_1, L_2 maps were recorded, are illustrated by the vertical dashed lines in Figs. 2(a) and 2(b). These L_1, L_2 maps showed a similar dependence of DPOAEs on stimulus levels as was described for Fig. 1: (1) for all stimulus frequencies, there was a distinction between the stimulus level dependence of low-level and high-level DPOAEs. This is illustrated by the convex shape of equal-amplitude contour lines for the low-level emissions, and the approximately straight contour lines for high-level emissions. (2) The low-level ridge, encompassed by the convex contour lines, ran

FIG. 2. Overview of the stimulus level dependence of DPOAE amplitude and phase for different stimulus frequencies. All data were obtained in a single ear using $f_2/f_1=1.1$. The left column gives DPOAEs at $2f_1-f_2$, while the right column gives DPOAEs at $2f_2-f_1$. (a)–(b) The DPOAE amplitude as a function of stimulus frequency f_1 obtained with $L_1=L_2=80$ dB SPL. The vertical dashed lines indicate the frequencies ($f_1=814, 1052, 1507, 1907$ Hz) for which L_1, L_2 recordings were made. The labels on the dashed lines refer to the subplots in the figure. (c)–(j) Amplitude and phase of DPOAEs as a function of stimulus levels L_1 and L_2 . The layout of the plots is similar to that in Fig. 1. That is, the DPOAE amplitude is represented by equal-amplitude contour lines, drawn at 3 dB intervals. The DPOAE amplitude is represented by shadings of grey, with the corresponding levels given in the key. The DPOAE phase is represented by the arrows overlying the contour lines, with a full counterclockwise rotation of the arrows corresponding to a phase lag of 2π rad. In each subplot, the phase is relative to the phase at $L_1=L_2=86$ dB SPL. As such, phase differences between the different subplots hold no meaning, but changes in phase within each subplot do.

approximately parallel to the diagonal $L_1=L_2$ for both $2f_1-f_2$ and $2f_2-f_1$. (3) The straight high-level contour lines had slopes equal to -2 dB/dB for $2f_1-f_2$ and -0.5 dB/dB for $2f_2-f_1$. (4) Within the low- and the high-level region of the L_1, L_2 maps DPOAE phase was relatively constant, but the transition between both regions was accompanied by a change in phase. Within each papilla, this change increased from close to 0 rad for low frequencies to almost π rad for high frequencies (see Fig. 3). Notice that in the AP the phase difference between low-level and high-level DPOAEs increases more rapidly with frequency than in the BP. A similar difference between the two papillae is found in the group delays of DPOAEs (Meenderink *et al.*, 2005). These differences in DPOAE phase behavior may signify differences in the mechanical filter responses of the two papillae.

Figure 2 shows another clear distinction between DPOAEs from the amphibian papilla and the basilar papilla. In the AP, low-level emissions resulted in an extensive plateau in the L_1, L_2 maps. In contrast, for DPOAEs in the BP this low-level plateau was much smaller or even absent.

The low-level ridge was not always on the diagonal $L_1=L_2$, but its position depends on the stimulus frequency relative to the peak frequency of the DPOAE audiogram. This can be illustrated most clearly for emission measurements at relatively large stimulus frequency ratios. Figure 4 displays results for one subject when $f_2/f_1=1.30$. At this ratio, only DPOAEs from the BP at $2f_2-f_1$ had an amplitude that was sufficient for a systematic evaluation. The DPOAE audiogram contains a single maximum at $f_1=1313$ Hz [Fig. 4(a)]. For a stimulus frequency f_1 at this maximum, the low-level ridge in the L_1, L_2 map is approximately on the diagonal $L_1=L_2$ [Fig. 4(c)]. However, when this stimulus frequency was on the low-frequency slope of the DPOAE audiogram, L_1 needed to be larger than L_2 to obtain a maximum emission amplitude. Consequently, the low-level ridge in the L_1, L_2 map was below the diagonal [Fig. 4(b)]. For a stimulus frequency above the maximum in the DPOAE audiogram, the reverse was true: L_2 needed to be larger than L_1 for a maximum emission amplitude, and the low-level ridge was above

FIG. 3. The difference in phase for DPOAEs at $2f_1-f_2$ (+) and $2f_2-f_1$ (o) evoked with $L_1=L_2=86$ dB SPL and $L_1=L_2=63$ dB SPL as a function of distortion product frequency. Data obtained with $f_2/f_1=1.02$ and 1.10 are combined. The vertical dashed line gives the frequency separation between the amphibian papilla and the basilar papilla (1250 Hz; Ronken, 1991).

FIG. 4. DPOAEs at $2f_2-f_1$ from the basilar papilla for $f_2/f_1=1.30$. (a) DPOAE amplitude as a function of stimulus frequency f_1 obtained with $L_1=L_2=80$ dB SPL. The vertical dashed lines give the frequencies ($f_1=1110, 1313, 1507$ Hz) for which L_1, L_2 recordings were made. The labels on these dashed lines refer to the subplots in the figure. (b)–(d) Amplitude and phase of DPOAEs as function of stimulus levels L_1 and L_2 . The diagonal dashed line in each subplot corresponds to $L_1=L_2$. The position of the low-level ridge relative to this diagonal depends on the stimulus frequency; compare subplots (b), (c), and (d). DPOAE amplitudes are coded in gray in 3 dB intervals, with the corresponding levels given in the key. The DPOAE phase is represented by the arrows overlying the contour lines, with a full counterclockwise rotation of the arrows corresponding to a phase lag of 2π rad.

the diagonal [Fig. 4(d)]. Nevertheless, for each stimulus frequency combination, the low-level ridge was parallel to the main diagonal $L_1=L_2$.

IV. DISCUSSION

It is currently believed that distortion product otoacoustic emissions (DPOAEs) from the frog arise from both the amphibian papilla (AP) and the basilar papilla (BP), the two end organs in the frog inner ear that are most sensitive to airborne sound. The bimodal shape of DPOAE audiograms is a direct result of this [Figs. 2(a) and 2(b); Van Dijk and Manley, 2001; Meenderink *et al.*, 2005]. In the present work we explored the dependence of DPOAE amplitude and phase on stimulus levels by systematically mapping L_1, L_2 space over a range of stimulus frequencies thus covering the frequency range of highest sensitivity of both papillae. The first obvious result is that across frequencies the L_1, L_2 dependence of DPOAEs is very different for high-level emissions compared to low-level emissions. In the presented L_1, L_2 maps, high-level DPOAEs were characterized by contour

FIG. 5. L_1, L_2 maps for three static nonlinear models. For each model, results are shown for the distortion product at $2f_1 - f_2$ (left column) and at $2f_2 - f_1$ (right column). Contour lines were calculated at 10 dB intervals. (a) Amplitude at $2f_1 - f_2$ for a cubic nonlinearity $y = x^3$. (b) Amplitude at $2f_2 - f_1$ for the same nonlinear model. (c) and (d) Results for the Boltzmann function $y = 1/(1 + \exp(-x))$. (e) and (f) Results for a Boltzmann function with a shifted operation point: $y = 1/(1 + \exp(-x + 3))$. The combination of the cubic nonlinearity [panels (a) and (b)] with a Boltzmann nonlinearity [panels (c) and (d)] may account for the L_1, L_2 maps recorded in the frog. The Boltzmann function with a shifted operation point may describe the transition from low- to high-level emissions. However, in that model the high-level contour lines do not match the approximately linear contour lines observed in the frog.

lines that followed (approximately) straight lines. In contrast, contour lines for low-level DPOAEs were clearly convex (e.g., Fig. 1). In mammals, L_1, L_2 maps show a similar difference between high-level and low-level DPOAEs (Mills *et al.*, 1993; Mills and Rubel, 1994; Whitehead *et al.*, 1995), while DPOAE I/O curves recorded in birds and reptiles indicate similar level-dependent differences in these vertebrate classes (Manley *et al.*, 1993; Kettembeil *et al.*, 1995; Taschenberger and Manley, 1998).

The shape of the contour lines reflects fundamental properties of the nonlinearity that underlies DPOAE generation. For a cubic nonlinear system,

$$y = x^3, \quad (1)$$

contour lines are straight [see Figs. 5(a) and 5(b)], similar to the high-level DPOAEs in both frogs and mammals. Small deviations from straight contour lines may occur for nonlinearities described by $y = \text{sign}(x)|x|^\nu$. For $\nu > 3$, contour lines

are concave while for $\nu < 3$ ($\nu \neq 1$) contour lines are convex. The slope of the contour lines for a cubic nonlinearity is $L_2/L_1 = -2$ dB/dB for $2f_1 - f_2$ and $L_2/L_1 = -0.5$ dB/dB for $2f_2 - f_1$, which is similar to the slopes of the high-level contour lines found here (e.g., Fig. 1).

If the nonlinearity has an (asymptotic) output clipping level, the contour lines have a shape that is markedly different. As an example, Figs. 5(c) and 5(d) show the L_1, L_2 dependence of distortion product amplitude for a Boltzmann nonlinearity,

$$y = \frac{1}{1 + e^{-x}}, \quad (2)$$

which clips to $y = 1$ for $x \rightarrow \infty$ and $y = 0$ for $x \rightarrow -\infty$. Here, the contour lines are convex, running parallel to the diagonal $L_1 = L_2$ in part of the L_1, L_2 space. Low-level DPOAEs from the frog had a dependence on the stimulus level, which is consistent with a clipping nonlinearity, such as the Boltzmann function.

In mammals, low-level contour lines are also convex, but, in general, the low-level ridge is not parallel to the diagonal $L_1 = L_2$ (Whitehead *et al.*, 1992; Kummer *et al.*, 2000; Pibal *et al.*, 2002). Kummer *et al.* (2000) and others interpreted this as being a manifestation of the differential growth of each stimulus amplitude at the presumed location of DPOAE generation, which results from the different growth of basilar membrane vibration at different locations along the cochlea (e.g., Ruggero and Rich, 1991). As such, it reflects the transformation of stimulus amplitudes at the tympanic membrane to stimulus amplitudes at the site of DPOAE generation.

In our results, the low-level ridge was parallel to $L_1 = L_2$, suggesting that the stimulus-induced vibration of the tectorial membranes in both the AP and the BP have the same growth rate. In other words, the tectorial membrane response may grow nonlinearly and compressively, but in contrast to the mechanics in the mammalian cochlea, this growth has to be the same for both stimulus frequencies. Note that the finding that the low-level ridge may be shifted away from $L_1 = L_2$ (Fig. 4) indicates that equal stimulus levels at the tympanic membrane not necessarily result in equal-amplitude vibrations in the tectorial membrane.

Typically, the observed level dependence of DPOAEs are interpreted as being the result of two level-dependent components (e.g., Kim, 1980; Whitehead *et al.*, 1992). Here, one component is dominant for high-level stimuli, while the second component dominates for low-level stimuli. Based on the differential vulnerability of the two components, it has been suggested that each component arises from a different mechanism (Mills *et al.*, 1993). In this two-source model the low-level component is linked to active hair cell mechanics, while the high-level component arises from a passive nonlinearity, possibly not associated with the hair cells.

Recently, doubt has risen whether the two-source model is applicable to DPOAE generation (Mills, 2002). Frolenkov *et al.* (1998) showed that chemical blocking of the outer hair cell motility abolishes both low-level and high-level DPOAEs in the guinea pig, while Mom *et al.* (2001) provided evidence that the high-level DPOAEs that remain after

cochlear ischemia are highly vulnerable to acoustic overstimulation. These results indicate that the high-level and low-level DPOAEs in mammals depend on active mechanisms. Model studies have shown that a properly configured, single Boltzmann nonlinearity can account for the nonmonotonicities and associated phase shifts seen in mammalian DPOAE I/O data (Lukashkin and Russell, 1998). When such models incorporate feedback they can also reproduce the differential vulnerability of low-level and high-level DPOAEs (Lukashkin *et al.*, 2002). Also, Mills (1997) proposed a model in which one type of active nonlinearity generates DPOAEs. The artificial separation of emissions generated at different locations along the basilar membrane results in the appearance of a “passive” and an “active” component that both arise from the same nonlinearity. In short, the combination of these results imply that both high-level and low-level DPOAEs may arise from one type of active nonlinearity.

However, Liberman *et al.* (2004) showed that mice lacking the protein prestin in their hair cell’s basolateral membrane (i.e., the presumed motor molecule of the cochlear amplifier is missing) do generate high-level DPOAEs, indicating that hair cell electromotility is not required for their generation. In addition, they found that after death these high-level DPOAEs disappear rapidly, with a time course similar to the post-mortem disappearance of the cochlear microphonic. Therefore, these “residual” high-level DPOAEs were clearly of biological origin and arose from a second active nonlinear mechanism that is perhaps related to the hair cell stereovillar bundles.

In a single Boltzmann nonlinear model, a transition from low- to high-level DPOAEs is accomplished by a shift of the operation point of the Boltzmann curve (Lukashkin and Russell, 1998). Regardless of the choice of operation point, the Boltzmann nonlinearity is a clipper for large stimulus amplitudes. Thus, a Boltzmann nonlinearity exhibits a transition from low to high levels, but the high-level contour lines are parallel to the main diagonal in the L_1, L_2 maps [see Figs. 5(e) and 5(f)]. This is in sharp contrast with our experimental findings [e.g., Figs. 1(a) and 1(b)]: the frog high-level contour lines were consistent with a cubic-nonlinear function [Eq. (1)]. Thus, we reject a single Boltzmann nonlinearity as a possible model for both low- and high-level DPOAE generation in the frog.

The applicability of the model proposed by Mills (1997) to the frog ear can be excluded *a priori*. Although it results in similar L_1, L_2 maps as in the frog ear, tonotopy is an essential part of the model. Since the basilar papilla is not tonotopically organized, its general applicability to the frog ear is excluded. Also, the frequency dependence of the phase shift between the low-level and the high-level DPOAEs (Fig. 3) suggests that two nonlinear mechanisms are involved in emission generation. These two mechanisms are somehow coupled by a dynamic element (e.g., a spring or a resistor).

There exist important differences in the growth of low-level DPOAEs from the AP and the mammalian cochlea on the one hand, and from the BP on the other hand. The extensive low-level plateaus in the AP indicate that the DPOAE amplitude grows compressively (<1 dB/dB) with increasing low-level stimuli, while at low levels growth in the BP was

expansive (>1 dB/dB). The Boltzmann nonlinearity results in expansive growth of the low-level distortion products, in accordance with the expansive growth of low-level DPOAEs in the BP (Meenderink and Van Dijk, 2004; this work). The Boltzmann nonlinearity has been proposed to describe the relationship between the hair bundle deflection and the transduction current in hair cells (Crawford *et al.*, 1989). Thus, DPOAE amplitude in the BP may be proportional to the transduction current in hair cells.

The compressive growth rate in the AP and the mammalian cochlea may be interpreted as being the result of an automatic gain control in these hearing organs. In other words, the generation of DPOAEs may be proportional to the transduction current in hair cells (described by the Boltzmann nonlinearity), but the measured DPOAE amplitude growth may reflect the additive effect of a compressive amplification mechanism.

In conclusion, our L_1, L_2 maps support the notion of two DPOAE-generation mechanisms in the frog. One component dominates for low-level stimuli, while the other is dominant for high-level stimuli. These components differ substantially with respect to their dependence on the stimulus levels, L_1 and L_2 . Combined with the finding that the phase difference between the two components varies with frequency, this suggests that different mechanisms underlie their generation.

ACKNOWLEDGMENTS

This work was supported by grants from the Netherlands Organization for Scientific Research (NWO), and the Heinsius Houbolt Foundation.

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