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van Dijk, P; Manley, GA

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Distortion product otoacoustic emissions in the tree frog *Hyla cinerea*

Pim van Dijk^{a,*}, Geoffrey A. Manley^b

^a Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Maastricht,
P.O. Box 5800, 6202 AZ Maastricht, The Netherlands

^b Institut für Zoologie, Technische Universität München, Lichtenbergstr. 4, 85748 Garching, Germany

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Abstract

The frog inner ear contains two hearing organs: the amphibian and the basilar papilla. The amphibian papilla is sensitive to low- and mid-frequency stimuli (0.1–0.5 and 0.5–1.3 kHz, respectively, in *Hyla cinerea*), while the basilar papilla is sensitive to high-frequency stimuli (2.8–3.9 kHz in *H. cinerea*). Distortion product otoacoustic emissions (DPOAE) were recorded from the ear of the tree frog *H. cinerea*. In each of six ears investigated, a cubic distortion product (DP) at $2f_1 - f_2$ was present when the primary frequencies f_1 and f_2 and the DP frequency were close to either the mid- or the high-frequency range. At frequencies between the sensitive ranges of both papillae, no emissions were observed. For the basilar papilla, the dependence of DP level on the primary tone frequency ratio f_2/f_1 showed a pattern characteristic of the response of a single nonlinear resonator. Thus, in agreement with neural data, DPOAE from the basilar papilla reflect the contribution of a single auditory filter to emission generation. © 2001 Elsevier Science B.V. All rights reserved.

Key words: Distortion product otoacoustic emission; Amphibia; Frog

1. Introduction

Various types of otoacoustic emissions have been found in all classes of terrestrial vertebrates (amphibia: Van Dijk et al., 1996; lizards: Köppl et al., 1993; Manley, 1997; birds: Taschenberger and Manley, 1998; mammals: Probst et al., 1991). Although not every category of emissions has been demonstrated in each particular species investigated so far, the very wide distribution of otoacoustic emissions among vertebrates favors the idea that the capacity to generate otoacoustic emissions is a very basic property of auditory hair cell epithelia (Köppl, 1995).

When two tones are played into the ear, distortion product otoacoustic emissions (DPOAE) can be measured in the external ear canal. In mammals, these

DPOAE originate both from those areas of the hearing organ that process the primary stimuli and from the area responsible for the frequency of the DPOAE (e.g. Martin et al., 1987; Whitehead et al., 1992). Humans listening to such pairs of pure tones perceive new frequencies that are not present in the eliciting stimulus (e.g. Smoorenburg, 1972). Such additional frequency components have been described at the level of auditory nerve fiber responses (Goldstein and Kiang, 1968; Kim et al., 1980), basilar membrane motion (Rhode and Cooper, 1993; Robles et al., 1991), the microphonic response (Gibian and Kim, 1982), the receptor potential of inner hair cells (Nuttall and Dolan, 1990), the hair cell bundle's motion (Jaramillo et al., 1993) and as distortion product otoacoustic emissions (DPOAE; Kemp, 1979). The frequencies of the DPOAE are determined by the frequencies of the generating primary tones, f_1 and f_2 ($f_2 > f_1$). Difference tones $f_2 - f_1$, summation tones $f_2 + f_1$ and combination tones $2f_1 - f_2$ and $2f_2 - f_1$ can usually be measured, and higher-order combination tones such as $3f_1 - 2f_2$ and $4f_1 - 3f_2$ have also been described.

Most studies have concentrated on the first lower

* Corresponding author. Tel.: +31 (43) 3877580;
Fax: +31 (43) 3875580; E-mail: p.van.dijk@kno.azm.nl

Abbreviations: AP-DP, amphibian papilla distortion product(s); BP-DP, basilar papilla distortion product(s); CF, characteristic frequency; DP, distortion product(s); DPOAE, distortion product otoacoustic emission(s); SOAE, spontaneous otoacoustic emission(s)

sideband distortion product ($2f_1 - f_2$), which is usually the most prominent distortion product measurable in the sound pressure in the external auditory meatus. Its level depends on the specific features of the eliciting stimuli, but is also very sensitive to the physiological state of the cochlea (e.g. Brown et al., 1989; Köppl, 1995; Lonsbury-Martin et al., 1987). Its level also correlates with the local auditory sensitivity. It has been shown for several species that the properties of this DPOAE reflect sound processing in the sensory epithelium over the whole hearing range. Thus, the maximum DPOAE emitted and the lowest thresholds of DPOAE detection are generally found in frequency ranges where auditory sensitivity is high for the species examined (Brown, 1987; Lonsbury-Martin et al., 1987; Manley and Köppl, 1993; Zurek et al., 1982).

Obviously, nonlinear processes in the inner ear are responsible for the generation of DPOAE (Markin and Hudspeth, 1995). Rhode (1971) first showed that the nonlinear behavior of the basilar membrane displacement as a function of sound pressure relies on the physiological integrity of the cochlea, i.e. this feature is not found in the dead animal. Moreover, the nonlinear stiffness of the hair cells' stereovillar bundle (Howard and Hudspeth, 1988; Jaramillo et al., 1993; Russell et al., 1992) could also be involved in DPOAE generation.

In comparison to the wealth of data on DPOAE in mammals (for review see e.g. Probst et al., 1991), there are fewer studies on this phenomenon in nonmammals. So far, DPOAE have been reported from lizards (alligator lizard: Rosowski et al., 1984; Taschenberger et al., 1995; bobtail lizard: Köppl et al., 1993; Manley and Köppl, 1992, 1993; Manley et al., 1990) and birds (chicken: Norton and Rubel, 1990; chicken and starling: Kettembeil and Manley, 1995; barn owl: Manley et al., 1999; Taschenberger and Manley, 1998).

In contrast to the universality of the presence of spontaneous otoacoustic emissions in all land vertebrate groups, DPOAE were reported to be absent in frogs (Baker et al., 1989). Since this might suggest a fundamental difference in the sound processing mechanisms at the hair cell level in amphibians as compared to amniotes (reptiles, birds and mammals), we decided to examine again the question of the existence of DPOAE in a frog species. We report here that DPOAE do, in fact, occur in amphibians. This is especially interesting with regard to questions concerning the place of origin of DPOAE, since frogs generally possess two different auditory papillae that process stimuli in divergent frequency ranges (Lewis et al., 1982a,b). Specifically, we describe data on the basic properties of the distortion product $2f_1 - f_2$ in the external ear canal of the green tree frog *Hyla cinerea*, such as the dependence of

DPOAE level on f_1 , on the frequency ratio and on the level of the primary tones.

2. Materials and methods

Distortion product otoacoustic emission were recorded in six tree frogs *H. cinerea*. In each frog, one ear was tested. Across animals, body weight ranged from 4.3 to 9.3 g (average 5.6 g), and snout-vent length ranged from 4.2 to 5.9 cm (average 4.9 cm). Animals were anaesthetized via immersion in 0.1% MS222 solution. During the experiment, an animal was covered by wet gauze soaked in the MS222 solution. In order to keep anesthesia light, we removed the gauze temporarily when all movement artefacts disappeared. Experiments were conducted in a sound-attenuating booth. Ambient temperature in the booth was between 23.0 and 24.3°C.

DPOAE were recorded using a probe placed around the frog's tympanic membrane. The probe contained a Beyer DT770 earphone for stimulus generation, and a Brüel and Kjær 4166 condenser microphone for emission recording. Stimuli were generated by two HP3325A function generators, each producing a sinusoidal signal. Both signals were adjusted by HP350D attenuators, mixed and subsequently fed to the Beyer earphone via a Klark-Teknik DN300 equalizer. The levels and frequencies of the stimulus tones (the 'primary' tones), will be denoted by L_1 , L_2 , f_1 , and f_2 , respectively. The primary frequency f_1 ranged from 421 to 5021 Hz. For each f_1 value, the frequency ratio f_2/f_1 was stepped from 1.05 to 1.5, while primary level ranged between 37 and 85 dB SPL. Only equal level primaries were used (e.g. $L_1 = L_2$).

The microphone (emission) signal was passed through a Brüel and Kjær 2660 low-noise preamplifier driven by a Brüel and Kjær 2804 power supply. The amplified microphone signal was processed using a HP 3561A Spectrum Analyzer. For each combination of primary levels and frequencies, 10 spectra were averaged by the spectrum analyzer.

Cubic distortion of this experimental system was at least 90 dB below the primaries, for stimulus levels up to $L_1 = L_2 = 100$ dB SPL.

The experimental procedure was controlled by a PC, which was connected to the tone generators and the spectrum analyzer via an IEEE/GPIB interface. The PC (a) controlled the amplitude and frequency of both stimulus tones, (b) recorded the spectral level of the microphone response at the distortion frequency $f_{dp} = 2f_1 - f_2$, and (c) estimated a noise level by averaging the spectral levels at 15 Hz on both sides of the distortion product frequency.

3. Results

Distortion product otoacoustic emissions were recordable in all six ears investigated, while five ears also emitted spontaneous otoacoustic emissions (SOAE), with frequencies ranging from 850 to 1530 Hz, and peak levels between 2 and 13 dB SPL.

Fig. 1 displays an example of the distortion emission spectrum for two primary tones in the frequency range of the basilar papilla ($f_1 = 3021$ Hz, $f_2 = 3323$ Hz). Besides the large peak corresponding to the primary tones, distortion products are visible at $3f_1 - 2f_2$, $2f_1 - f_2$, and $2f_2 - f_1$. Similar spectral analysis for primary tones in the amphibian papilla frequency range ($f_1 = 1021$ Hz, $f_2 = 1123$ Hz, $L_1 = L_2 = 60$ dB SPL) showed distortion products at $4f_1 - 3f_2$, $3f_1 - 2f_2$, $2f_1 - f_2$, $2f_2 - f_1$, $3f_2 - 2f_1$, and $4f_2 - 3f_1$. In the current study, only the level of distortion product at $2f_1 - f_2$ was systematically recorded.

Following the convention used to represent DPOAE data in the lizard (Köpl et al., 1993), we will describe DPOAE level as function of the primary frequency f_1 . As a function of frequency f_1 , the cubic distortion product level showed a bimodal dependence: the amplitude response resembles that of two bandpass filters in parallel, one approximately centered at 1 kHz, the other at about 3 kHz. As an example, Fig. 2 shows data for one ear. For clarity we will refer to the two ‘modes’ as amphibian papilla distortion products (AP-DP) and basilar papilla distortion products (BP-DP), respectively (see Section 5). We will characterize both modes by (1) the primary frequency for which the DPOAE level was maximal, i.e. the center frequency, (2) low- and high-side cutoff frequencies for which the DPOAE level was 10 dB attenuated with respect to its maximum, (3) a quality factor $Q_{10\text{dB}}$ (as follows from (1) and (2)), and

Fig. 1. Spectrum of the microphone signal, showing DPOAE peaks from the basilar papilla of *H. cinerea*; $L_1 = L_2 = 70$ dB SPL, $f_1 = 3021$ Hz, $f_2 = 3323$ Hz. The spectral peaks corresponding to the primary tones exceed the vertical scale (arrows). Combination tone peaks are labelled by their frequency: $3f_1 - 2f_2$, $2f_1 - f_2$, and $2f_2 - f_1$.

Fig. 2. (a) Solid curves: levels of the $2f_1 - f_2$ distortion product as a function of primary frequency f_1 . Ratio $f_2/f_1 = 1.1$. Primary levels: squares, $L_1 = L_2 = 46$ dB SPL; up triangles, 51 dB SPL; diamonds, 56 dB SPL; down triangles, 61 dB SPL; circles, 66 dB SPL. Thin dashed curves: noise levels. (b) Spectrum of microphone signal while no stimulus was present for the same ear. Two spontaneous otoacoustic emission peaks are visible (arrows).

finally (4) the low- and high-side slopes in dB per decade increase of the primary frequency f_1 . For each parameter, we will indicate ranges observed across subjects. The cutoff frequencies were determined by linear interpolation from the actually measured frequency values.

Fig. 3. DPOAE level for $L_1 = L_2 = 70$ dB SPL for different f_2/f_1 ratios. Each ear is represented by a particular symbol. All ratios f_2/f_1 from 1.05 to 1.5 are included.

Fig. 4. (a) Level of the $2f_1 - f_2$ distortion product as a function of the ratio f_2/f_1 . Results for both the amphibian papilla (closed symbols) and the basilar papilla (open symbols) are shown. Each curve is marked by the value of the corresponding primary frequency f_1 . For $f_1 > 3000$ Hz an optimum ratio is apparent. With increasing frequency f_1 , the optimum ratio increases. (b) Same data as in panel (a), but plotted as a function of the $2f_1 - f_2$ distortion product frequency. Symbols and line styles correspond to panel (a). Again, each curve corresponds to a particular primary frequency f_1 , while f_2 , and thus $2f_1 - f_2$ was varied. The curves corresponding to a $f_1 > 3000$ Hz align, with their optimum at a characteristic frequency (CF). The CF of this basilar papilla is 2.6 kHz (fine dashed line). Plots (c) and (d): Same as (a) and (b), respectively, but for a Duffing oscillator tuned to CF = 3 kHz (see Section 4). The Duffing oscillator exhibits a behavior similar to the basilar papilla: for $f_1 > CF$, an optimum ratio is observed, which increases with increasing f_1 . When plotted as a function of the distortion product frequency, these optima align (panel (d)).

The center frequency for AP-DP was $f_1 = 921$ to 1421 Hz. The 10 dB cutoff frequencies were 466 to 855 Hz and 1197 to 1488 Hz, respectively. This corresponds to a filter quality factor $Q_{10\text{dB}} = 1.2$ to 1.6 . The increase of DPOAE level with frequency was relatively shallow on the low-frequency side (11 to 14 dB/octave). In contrast,

on the high-frequency side, the decrease of DPOAE level with increasing frequency of the primaries was much steeper (-39 to -87 dB/octave).

For BP-DP the center frequency was between $f_1 = 2621$ and 3221 Hz. The 10-dB cutoff frequencies ranged from 1652 to 2832 and 3342 to 3669 Hz, respec-

tively. This corresponds to quality factors from 1.6 to 3.8. Both for the low frequency and the high frequency slope of the basilar papilla, a steep increase/decrease was observed: 42 to 84 dB/octave and -36 to -78 dB/octave.

The DPOAE growth rate with level of the pure tone stimulus ranged from 0.5 to 1.9 dB/dB, with an average of 0.90 dB/dB, for AP-DP, and from 0.4 to 1.3 dB/dB, with an average of 0.85 dB/dB for BP-DP. For primary tone levels $L_1 = L_2 = 70$ dB SPL, the DPOAE levels ranged from -3 to 25 dB SPL (Fig. 3). Typically, DPOAE levels were lower for the basilar papilla than for the amphibian papilla, but in one case the basilar papilla emitted the stronger emissions (Fig. 3, down triangles).

The DPOAE level was dependent on the ratio f_2/f_1 . For the amphibian papilla, DPOAE level either decreased or was approximately constant with increasing ratio. On the low-frequency side of the papilla, with f_1 below 700 Hz, the DPOAE typically decreased with increasing ratio, and disappeared in the noise floor (0 dB SPL in that frequency range) for ratios above 1.25. For higher primary frequencies, the DPOAE level was approximately constant up to $f_2/f_1 = 1.3$ – 1.4 , and then decreased (see Fig. 4a, closed symbols, for a typical example).

Basilar papilla DPOAE displayed a characteristic level dependence on the primary tone ratio f_2/f_1 (see Fig. 4a). For primary frequencies f_1 below the characteristic frequency (CF), the ratio dependence resembled that of the upper half of the amphibian papilla: with increasing frequency, DPOAE level was approximately constant, and dropped for ratios above 1.2– 1.4 . If f_1 was above the papilla's CF, an optimum ratio was observed. This optimum ratio was larger for higher-frequency f_1 (see Fig. 4a).

The same data are plotted again in Fig. 4b, but as a function of the distortion product frequency $2f_1 - f_2$. Again, an optimum is evident when $f_1 > \text{CF}$. The optima align when the curves are plotted as a function of distortion product frequency, with the distortion product largest when it coincides with CF.

The distortion product level seemed to be dependent on the depth of anesthesia. The depth of anesthesia was assessed by the presence of movement artefacts in the microphone signal. In three frogs, all movement artefacts disappeared during the experiment. This was correlated with the disappearance of DPOAE and SOAE. In these cases, the MS222 soaked gauze was temporarily removed from the animal's back. This resulted in a partial recovery from the anesthesia, which correlated with the reappearance of DPOAE and SOAE. Since this experiment was targeted to DPOAE, we did not assess whether DPOAE and SOAE reappeared simultaneously.

4. Theory: the Duffing oscillator

As a simple model of distortion product otoacoustic emissions in the basilar papilla, we will consider the Duffing oscillator (Duffing, 1918; Guckenheimer and Holmes, 1983). The Duffing oscillator describes a second-order resonance with a parabolic nonlinear stiffness. These components are simplified representations of the high-order filter mechanism of the basilar papilla (Van Dijk et al., 1997), and the nonlinear stiffness of the hair bundles of frog hair cells (Jaramillo et al., 1993). Although the Duffing oscillator is obviously a simplification of basilar papilla mechanics, it is expected to qualitatively describe some characteristics of DPOAE in frogs. In particular, the relation between primary frequency f_1 and the optimum frequency ratio f_2/f_1 (Fig. 4a and b) is expected to be qualitatively described by the Duffing oscillator.

The Duffing oscillator is described by the nonlinear second-order equation

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

where m is a mass, whose movement is driven by an external force $F(t)$. The linear resistance R impedes the movement, while a nonlinear stiffness $k(x)$ tries to restore the mass' position x to its equilibrium position $x = 0$. The Duffing oscillator is characterized by the parabolic stiffness function

$$k(x) = k_0 \left(1 + \frac{x^2}{x_0^2} \right). \quad (2)$$

Note that the stiffness increases for increasing deviation of the mass from the equilibrium position.

When the oscillator is driven by a 'two-tone' force

$$F(t) = A_1 \sin 2\pi f_1 t + A_2 \sin 2\pi f_2 t \quad (3)$$

cubic distortion products are present in the response $x(t)$. We simulated the oscillator behavior using a fourth-order Runge–Kutta integration method (Press et al., 1992), and considered the amplitude of the cubic distortion product $2f_1 - f_2$ as a function of the ratio f_2/f_1 . Fig. 4c shows results for $m = 1$, $r = 2 \cdot 10^3 \text{ s}^{-1}$, $k_0 = (2\pi \times 3000)^2 \text{ s}^{-2}$, $x_0 = 7 \cdot 10^{-8}$, and $A_1 = A_2 = 1$. If f_1 is placed below the oscillator's resonance frequency $(1/2\pi)\sqrt{k_0/m} = 3000 \text{ Hz}$, the distortion product level does not show a pronounced ratio dependence. In contrast, for frequencies $f_1 > 3000 \text{ Hz}$, above the resonance frequency, a clear optimum ratio is present. The optimum ratio is larger for higher primary frequency f_1 . This is further illustrated in panel (d) of Fig. 4, where the simulation results are shown as a function of distortion product frequency. As can be seen, the optimum

ratio corresponds to a situation where the distortion frequency is at the resonance frequency of the oscillator (3000 Hz, fine dashed line).

5. Discussion

This is the first report which shows the presence of distortion product otoacoustic emission (DPOAE) in amphibians. With the finding that DPOAE are emitted by the amphibian inner ear, DPOAE have now been reported in all land vertebrate groups: mammals, birds, reptiles and amphibians.

In contrast to our results, Baker et al., 1989 did not find DPOAE in the frog species, *Rana temporaria*, *Rana pipiens*, and *Rana esculenta*. This contrasts with the finding that spontaneous otoacoustic emissions have a similar range of sound pressure levels for Ranidae and Hylidae (Van Dijk et al., 1996). In addition, the minor structural and functional differences between both frog families are not expected to account for the absence of DPOAE in the Ranidae. Nevertheless, the reported absence of DPOAE in Ranidae (Baker et al., 1989) may reflect species differences. Also, it may reflect anesthesia problems (see below).

Baker et al. (1989) found no DPOAE exceeding instrumental distortion, for a 'wide range' of primary frequencies (Wilson et al., 1990). Instrumental distortion was at 10 dB SPL, for 80 dB SPL stimuli. For 70 dB stimuli, we report DPOAE up to 25 dB SPL. Obviously, these DPOAE would have been detected with the equipment used by Baker et al. (1989). Consequently, the failure to find DPOAE in Ranidae cannot be the result of limitations in the equipment used by these authors.

Alternatively, the absence of DPOAE in Ranidae may be related to the anesthesia procedures used by Baker et al. (1989). They injected an MS222 solution i.p., while we used immersion in an MS222 solution. The first method may have resulted in a deeper anesthesia, which may have suppressed DPOAE. However, Baker et al., 1989 did detect SOAE, despite the absence of DPOAE. This contrasts with our finding that both DPOAE and SOAE disappeared during deep anesthesia. Consequently, anesthetic effects may have contributed to the results of Baker et al. (1989), but a full understanding of the absence of DPOAE in the Ranidae remains obscure.

The bimodal dependence of DPOAE level on the primary frequency f_1 (see Fig. 1) corresponds to the bimodal distribution of characteristic frequencies of primary auditory nerve fibers. Low- and mid-frequency nerve fibers originate from the frog's amphibian papilla, while high-frequency fibers contact the basilar papilla (Lewis et al., 1982a,b). In *H. cinerea*, low- and mid-

frequency fibers are tuned to frequencies in the range 0.1–1.3 kHz, while high-frequency fibers are tuned to 2.8–3.9 kHz (Ehret and Capranica, 1980; Capranica and Moffat, 1983). Thus, a bimodal distribution is present in both nerve fiber and DPOAE characteristics. Consequently, it is straightforward to assume that lower-frequency DPOAE originate from the amphibian papilla, while higher-frequency DPOAE are generated in the basilar papilla. These two 'modes' of distortion products are therefore referred to as amphibian papilla distortion products (AP-DP) and basilar papilla distortion products (BP-DP). We will separately discuss the properties of AP-DP and BP-DP.

The amphibian papilla consists of a strip of hair cells embedded in a relatively stiff structure, and covered by a specialized tectorial membrane. The papilla displays a tonotopic organization, with the highest frequencies (1.3 kHz in *H. cinerea*) being represented caudally (Lewis et al., 1982a,b). AP-DP were largest between 0.47 kHz and 1.5 kHz. Apparently, the low-frequency part of the amphibian papilla, which is sensitive down to 0.1 kHz, does not contribute to emission generation. In contrast to the caudal higher-frequency portion of the papilla, tuning in the rostral portion is based on electrical resonance of sensory hair cells (Pitchford and Ashmore, 1987; Smothermann and Narins, 1999a). Thus, the absence of low-frequency DPOAE in *H. cinerea* may be related to the electrical tuning mechanism. However, DPOAE have been recorded in the bobtail lizard and the barn owl (Köppl et al., 1993; Taschenberger and Manley, 1998) in the frequency range which presumably relies at least partly on electrical tuning. Consequently, the absence of DPOAE from the electrically tuned area of the amphibian papilla in *H. cinerea* does not reflect a principle characteristic of electrical hair cell tuning.

An alternative explanation for the absence of low-frequency DPOAE in *H. cinerea* may be related to the mechanics of the tectorial membrane. The rostral low-frequency patch of the amphibian papilla is covered by a rather bulky tectorium, which may impede outward travel of a possible distortion product generated in the hair cells. Note that the low-frequency slope of the AP-DP was 11–14 dB/octave, e.g. may reflect rather simple second-order (12 dB/octave, high-pass) mechanics of the tectorium on the low-frequency portion. Thus, the absence of low-frequency DPOAE in *H. cinerea* may reflect a high-pass filtering of the tectorium in the rostral part of the amphibian papilla.

The high-frequency 10-dB cutoff of AP-DP was observed for $f_1 \approx 1.5$ kHz, corresponding to a distortion product frequency $f_{2f_1-f_2} \approx 1.35$ kHz (for $f_2/f_1 = 1.1$). Given the slope of 35 dB/octave of neural tuning curves, this approximately corresponds to the 10-dB upper cutoff frequency of the amphibian papilla

(1.3 kHz). Thus, the upper frequency portion of the amphibian papilla presumably generates AP-DP. Interestingly, it has been suggested that the upper (rostral) portion of the papilla supports a travelling wave, similar to the travelling wave in the mammalian cochlea (Hillery and Narins, 1984).

The range of AP-DP frequencies approximately corresponds to the range of SOAE frequencies (0.45–1.6 kHz: Van Dijk et al., 1989; Long et al., 1996; Van Dijk et al., 1996). This strongly suggests that SOAE are generated in the amphibian papilla, despite the fact that SOAE frequencies somewhat exceed the amphibian papilla neural CF range.

DPOAE from the basilar papilla (BP-DP) are of particular interest. The basilar papilla is a simple auditory receiver. Like the amphibian papilla, it consists of a small patch of sensory hair cells covered by a tectorial membrane (Frishkopf and Flock, 1974). In *H. cinerea*, all hair bundles are oriented in the same direction. In contrast to the amphibian papilla, in a single subject, nearly all nerve fibers contacting the basilar papilla have virtually identical tuning characteristics (Ronken, 1990, 1991), while some fibers are tuned to a higher frequency than the main bulk of fibers (Van Dijk et al., 1997). Thus, the basilar papilla mainly functions as a single auditory filter. Consequently, the BP-DP we describe reflect the contribution of a single auditory filter.

We will discuss the properties of BP-DP in relation to tuning of nerve fibers from the basilar papilla. This comparison is relevant since both neural and DPOAE tuning presumably originate from the same mechanism, as is clear from two considerations: (1) The neural tuning of basilar papilla auditory nerve fibers presumably originates from a mechanical tuning mechanism (Smothermann and Narins, 1999b). Since the frog basilar papilla lacks a basilar membrane, and the hair cells are embedded in a stiff structure, it is straightforward to assume that mechanical tuning results from the mechanics of the hair bundles and the tectorial membrane. (2) The BP-DP are probably generated by nonlinear mechanics of the basilar papilla hair cells. Any otoacoustic emissions generated by the basilar papilla will be filtered by the mechanics of the hair bundles and tectorial membrane. Combining these two arguments leads to the conclusion that BP-DP and neural tuning most probably reflect the same filter mechanism.

As a function of primary frequency, BP-DP display a filter function with tuning characteristics similar to basilar papilla neural tuning curves: the quality factor Q_{10dB} and the center frequency are similar to values reported for neural responses (reviewed in Ronken, 1991). Nevertheless, the range of tuning frequency we report for DPOAE (2.6–3.2 kHz) is somewhat lower than the range observed for neural characteristic fre-

quencies (2.8–3.9 kHz; Ehret and Capranica, 1980; Capranica and Moffat, 1983). Also, both neural studies report minor differences: while Ehret and Capranica (1980) found characteristic frequencies to be 2.8–3.8 kHz, Capranica and Moffat (1983) report 3.2–3.9 kHz. Small population differences among the various studies (including ours) may possibly account for these findings.

The dependence of BP-DP level on the pure tone ratio f_2/f_1 displays a characteristic pattern. Because the basilar papilla functions as a single auditory filter, we modelled its DPOAE response with a Duffing oscillator, which consists of a single resonance with nonlinear stiffness characteristics. The Duffing oscillator qualitatively describes the dependence of DPOAE level on pure tone ratio: when the primary tone frequency f_1 is above the resonance frequency of the resonator, the distortion product level displays an optimum at a particular ratio. This optimum ratio increases with increasing tone frequencies f_1 .

For the Duffing oscillator, the optimum ratio corresponds to the case where the distortion product frequency $2f_1 - f_2$ approximately coincides with the oscillator's resonance frequency (see CF in Fig. 4d). For BP-DP, the optimum ratio corresponded to a DP frequency between 2.0 and 2.8 kHz (2.6 kHz for the animal displayed in Fig. 4b). Again, this is somewhat below the range of neural characteristic frequencies (2.8–3.9 kHz; Ehret and Capranica, 1980; Capranica and Moffat, 1983). Possibly, driving the basilar papilla by two pure tones above its resonant frequency shifts its resonant frequency down. This behavior can probably be modelled by a more realistic oscillator model of the basilar papilla, which, however, requires systematic assessment of other DPOAE from the basilar papilla (e.g. $3f_1 - 2f_2$, $2f_2 - f_1$, $3f_2 - 2f_1$, etc.).

6. Conclusions

DPOAE from the amphibian inner ear are reported for the first time. Thus, DPOAE have now been reported in all classes of land vertebrates: amphibians, lizards, birds and mammals.

We discussed DPOAE in the tree frog *H. cinerea* in relation to the neural tuning properties of both hearing organs in the frog inner ear: the amphibian papilla and basilar papilla. DPOAE from the *amphibian* papilla originate from the caudal part of the papilla, i.e. the part in which frequency selectivity does not originate from electrical tuning but rather mechanical. DPOAE from the *basilar* papilla, which mainly functions as a single auditory filter, qualitatively resemble the response of a nonlinear single resonance system (e.g. a Duffing oscillator).

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