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Distortion product otoacoustic emissions in frogs: correlation with middle and inner ear properties

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Abstract

Four frog species, *Rana pipiens*, *Scaphiopus couchii*, *Xenopus laevis* and *Bombina orientalis*, were examined for distortion product otoacoustic emissions (DPOAE). These species were chosen for their diverse otic morphologies. *Rana* has a well-developed caudal extension of the amphibian papilla within the inner ear; *Scaphiopus*, *Xenopus* and *Bombina* do not. *Rana* and *Scaphiopus* have a tympanic middle ear, *Xenopus* has a subcutaneous tympanic disk and *Bombina* has only an operculum. DPOAEs were present in *Rana* and *Xenopus*, with amplitudes up to 55 and 20 dB SPL, respectively. DPOAEs could be detected in neither *Scaphiopus* nor *Bombina*. These results show that (1) a well-developed caudal extension is not necessary for generation of DPOAEs, and (2) a tympanic middle ear is neither required nor sufficient to have DPOAEs.

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Key words: Distortion product otoacoustic emission; Hearing; Amphibia; Frog; Inner ear

1. Introduction

The inner ear performs a similar function in all vertebrates: it separates sound into its frequency components and transduces the individual components into electrical activity of nerve fibers. Consequently, the nerve fibers in the auditory nerve respond in a frequency-selective manner and provide the brain with both spectral and temporal information on sound from the environment. Currently, it is believed that the frequency selectivity of the inner ear is supported by an active feedback mechanism (Gold, 1948; Davis, 1983), in which the sensory hair cells amplify auditory

stimuli and are able to acoustically (i.e. mechanically) drive the inner ear structures. The primary evidence for an active mechanism in the inner ear is the presence of spontaneous otoacoustic emissions (SOAEs; Kemp, 1979; Wilson, 1980; Zurek, 1981).

Otoacoustic emissions (OAEs) can also be recorded in response to an auditory stimulus. Evoked OAEs are typically classified with respect to the method of stimulation (Probst et al., 1991). Here, we will describe distortion product otoacoustic emissions (DPOAEs). DPOAEs are acoustic intermodulation distortion products (DPs) which are generated in the ear in response to two pure tones. The generation of DPs does not necessarily imply an active process in the inner ear. However, since SOAEs, DPOAEs and other evoked emissions show related variations with stimulus frequency ('fine structure', reviewed in Talmadge et al., 1998), their generation mechanisms presumably are closely related.

OAEs have been recorded in all terrestrial vertebrate classes and share several properties across species (reviewed in Köppl, 1995): (1) OAEs can be suppressed by an external tone. This suppression can be characterized by a suppression tuning curve. In all species investi-

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Abbreviations: DP, distortion product; DPOAE, distortion product otoacoustic emission; SOAE, spontaneous otoacoustic emission; OAE, otoacoustic emission

gated, this suppression tuning curve closely resembles neural tuning curves; (2) in all species, OAE properties are temperature-dependent; (3) OAEs are physiologically vulnerable in that they are sensitive to drugs and acoustic overstimulation. The presence and similarity of OAEs across species suggests a common generation mechanism.

Two possible mechanisms have been proposed for OAE generation. In the mammalian ear, outer hair cells display somatic movements in response to electrical stimulation (Brownell et al., 1985). Such somatic movements occur over a frequency range well exceeding the auditory range (Frank et al., 1999) and could potentially result in emission of sound via the middle ear. Two findings argue against the involvement of somatic hair cell movements in OAE generation. Firstly, somatic movements occur in in vitro preparations of isolated hair cells and must be considered to be physiologically robust. This is in stark contrast to the vulnerability of OAEs. Secondly, somatic length changes have only been observed in mammalian hair cells and are unlikely to be present in non-mammalian species (He et al., 2001; Manley, 2001). In other words, while somatic length changes may play a role in mammalian emission generation, they cannot be responsible for OAEs in all vertebrates.

An alternative mechanism for OAE generation is hair bundle movement. Such movements have been observed in the frog sacculus (Martin and Hudspeth, 1999), but given the fact that the transduction mechanisms in various animal species and hair cell types are similar, bundle movements may well be present across species and at auditory frequencies.

In the current paper, we do not directly address the OAE generation mechanism, but rather try to understand why OAEs are absent in some frog species. Van Dijk et al. (1996) showed that SOAEs are present in *Rana pipiens*, *Hyla cinerea*, *Hyla chrysoscelis*, *Hyla versicolor* and *Leptodactylus albilabris* but were not detected in *Xenopus laevis* or *Bombina orientalis*. These workers put forward two hypotheses regarding the absence of OAE in *Bombina* and *Xenopus*. Their first hypothesis states that the absence of OAE in these species is due to their non-tympanic middle ears. The rationale behind this first hypothesis is that if sound in the inner ear cannot be transmitted out via a tympanic membrane, no OAEs are detectable. Both *Xenopus* and *Bombina* lack a tympanic membrane. Instead, *Xenopus* possesses a subcutaneous, cartilaginous tympanic disc, coupled to the inner ear by means of the stapes: this arrangement is apparently adapted to underwater hearing (Wever, 1985; Elepfandt et al., 2000). The middle ear apparatus of *Bombina* is very reduced, lacking tympanic membrane, middle ear cavity and a functional stapes: sound seems to reach the inner ear in this spe-

cies via the lungs (Hetherington and Lindquist, 1999). Van Dijk et al. (1996) tried to measure OAEs from these animals by placing the microphone probe over the otic region behind the eye. According to this first hypothesis, any sound from the inner ear would be considerably attenuated before it reached the microphone, and might therefore be undetectable.

Van Dijk et al.'s second hypothesis on the absence of SOAEs in *Bombina* and *Xenopus* is related to the structure and function of the amphibian papilla in the inner ear. The amphibian papilla is one of the several end-organs of the frog inner ear and is responsible for the transduction of low-frequency airborne and seismic vibratory stimuli. SOAEs are presumably generated in the amphibian papilla rather than in the basilar papilla (Van Dijk and Manley, 2001). In *Rana* and *Hyla*, which have SOAEs, the amphibian papilla reaches its most elaborated form among frogs. In contrast, in *Bombina* and *Xenopus* it is considerably shorter (Lewis, 1984). As a second hypothesis, Van Dijk et al. (1996) suggested that a reduction of the caudal extension of the amphibian papilla might attenuate or preclude emission generation.

In order to test the two hypotheses, we recorded OAEs in four frog species: *Rana pipiens*, *Scaphiopus couchii*, *Xenopus laevis* and *Bombina orientalis*. The anatomical variations by which these species differ (see Table 1) allowed us to assess various factors which are important in emission generation. Emission recording was primarily targeted towards determining whether emissions are present in a particular species. We measured DPOAEs rather than SOAEs. Across species, the incidence of DPOAEs is higher than or equal to that of SOAEs (Probst et al., 1991; Köppl, 1995; frog: Van Dijk and Manley, 2001). Rather than measuring SOAEs, determining the presence or absence of DPOAEs in a particular frog species is probably a more stringent test of the presence or absence of an emission-generating mechanism in the inner ear. Since the middle ear is important in transmitting sound (i.e. emissions), we additionally determined the motion of the tympanic membrane, or skin overlying the otic region, in response to sound. A pronounced movement of the tympanic membrane or skin suggests a role of the middle ear as a sound path to and from the inner ear.

2. Materials and methods

The animals included in this study were as follows: Northern leopard frog (*Rana pipiens pipiens*): $n=9$ males and $n=1$ female, body mass 22.0–45.7 g, snout–vent length 64.0–81.4 mm, purchased from W.M.A. Lemberger Co. (Oshkosh, WI, USA); African clawed frog (*Xenopus laevis*): $n=2$ males, body mass

Table 1
Anatomical properties of the four frog species studied (Wever, 1985; Lewis, 1981)

	Tympanic disk or membrane	Caudal extension length beyond tectorial curtain
<i>Bombina</i>	absent	0 μ m
<i>Xenopus</i>	disk	100–150 μ m
<i>Scaphiopus</i>	membrane	100–150 μ m
<i>Rana</i>	membrane	> 200 μ m

The key properties listed in the table are those which are important for testing the two hypotheses forwarded by Van Dijk et al. (1996).

32–34 g, snout–vent length 60.9–65.2 mm, from *Xenopus* Express (Homosassa, FL, USA); Oriental fire-bellied toad (*Bombina orientalis*): $n=1$ male, body mass 8.6 g, snout–vent length 47.7 mm, from Allan's Aquarium and Pet Center (Venice, CA, USA); and Couch's spadefoot toads (*Scaphiopus couchii*): $n=4$ males and $n=5$ females, body mass 7.8–32.3 g, snout–vent length 39.7–62.0 mm, collected in Cochise County, AZ, USA on 19–20 September 2000, under Scientific Collecting Permit number SP582436 issued by the State of Arizona Game and Fish Department.

Animals were anesthetized with an intramuscular injection of pentobarbital sodium solution (Nembutal, Abbott Laboratories, 50 mg/ml: 1.0–1.3 μ l/g body mass), with supplementary doses given as necessary. The mouth cavities of *Rana*, *Scaphiopus* and *Xenopus* were examined prior to emission recording to ensure that the Eustachian tubes were free of mucus.

OAE measurements were performed in a sound-attenuating chamber. Emissions were recorded with an ER10C probe (Etymotic Research, Elk Grove, IL, USA), which includes a sensitive microphone and two miniature speakers. This system is designed to be used with human subjects, and comes with ear tips to be inserted into the ear canal. Since there is no ear canal in the frog species studied, we placed a small, plastic tube (inner diameter 9.5 mm, length 21 mm) over the ear tip. In *Rana* and *Scaphiopus*, the probe assembly was coupled to the frog ear by placing the open end of the tube on the skin around the tympanic membrane. In *Xenopus* and *Bombina*, no tympanic membrane is present. In *Xenopus*, the plastic tube was placed in a position estimated to correspond with the center of the tympanic disc, which is buried under soft tissues caudal to the eye. In *Bombina*, the plastic tube was positioned on the lateral otic region behind the eye. In all cases, a closed acoustic delivery system was achieved by sealing the space between the tube and the skin with silicone grease. The total volume in the closed space in the tube of the probe assembly was 0.7 cm³.

In order to measure DPOAEs, the miniature speakers built into the ER10C probe were used to stimulate the

frog ear with two primary tones with frequencies f_1 and f_2 , respectively, where $f_2/f_1=1.1$. For each frog, DPOAEs were recorded for $210 \leq f_1 \leq 3010$ Hz, with a 100-Hz resolution. Tone levels were $L_1=L_2=88$ (± 2) dB SPL for stimulus frequencies above 1 kHz. Due to limitations of the miniature speakers, primary tone levels were somewhat lower below 1 kHz, and decreased by 8 dB/octave down to $70(\pm 6)$ dB for the lowest stimulus frequency $f_1=210$ Hz. The two sinusoidal signals which were used to drive the speakers were generated using WG1 waveform generators (Tucker Davis Technologies, Gainesville, FL, USA). The level of each tone was adjusted with a programmable attenuator (PA4; Tucker Davis Technologies).

The microphone signal was amplified using the amplifier provided with the ER10C and fed to a spectrum analyzer (Stanford Research Systems SR770). The bin-width of the spectra was 1.95 Hz. For each setting of the primary tones a mean spectrum was calculated, by averaging the spectra of 16 consecutive 50% overlapping time windows, which corresponds to a total measuring time of 4.35 s. For each averaged spectrum the levels at frequencies f_1 , f_2 , $2f_1-f_2$ and $2f_2-f_1$ were stored.

The noise floor and nonlinear distortion of the DPOAE recording setup were determined by performing control measurements with the probe pressed against a hard surface.

In addition to DPOAE recordings, we performed laser measurements of tympanic membrane or otic skin motion in response to sound. These measurements were performed with the frog placed on a vibration-isolated table (Backer-Loring Micro-g) within a double-walled sound-attenuating chamber (IAC-1202). The inner walls of the chamber were covered with foam.

Free-field auditory stimuli were presented via a 10 cm speaker (Analog and Digital Systems, type 300) positioned on the left side of the frog with its cone 75 cm from the center of the frog's interaural axis, at an azimuth of 30° to the midline and an elevation of 13°. Two-second duration pure tones, from 150 Hz to 3 kHz in 30 Hz steps, were synthesized by a custom program (Acoustic Analyzer by A. Purgue, 1999) running on an Apple Macintosh iMac computer. The output of the computer's 16-bit D/A board was amplified (Optimus, type MPA-50) and sent to the speaker. The sound level near the frog's head was calibrated with a probe microphone (Knowles Electronics, type EK-3033), connected to the computer's A/D board. Using the calibration results, the computer adjusted the stimulus level at the animal's eardrum to 90 dB SPL for each stimulus frequency.

The tympanic membrane response was measured using laser Doppler vibrometry. Since the skin of the animals was not reflective enough for the laser measure-

Fig. 1. DPOAE spectrum in *Rana pipiens*. The two largest peaks correspond to the stimulus tones at frequencies $f_1 = 1210$ Hz and $f_2 = 1.1 \times f_1 = 1331$ Hz, respectively. The DPOAE amplitudes at $2f_1 - f_2$ and $2f_2 - f_1$ were systematically recorded in this study. Additional DPOAE peaks were present (arrows).

ments, a small, square mat of reflective beads mounted on adhesive backing material (approximately 0.1×0.25 mm², mass 20–45 μ g) was positioned on the structure to be measured. One bead mat was placed on the center of the tympanic membrane (or skin of the otic region in *Xenopus* and *Bombina*), and another on the shoulder region as a control. The frog was positioned so that the center of the bead mat was placed in the beam of a He–Ne laser, emitted from a single-point interferometer sensor head (Polytec, type OFV-303).

Due to limitations of the laser setup, only structures on the left side of the animal could be easily measured. Consequently, we chose to limit the DPOAE recordings to left ears as well, except in the single *Bombina orien-*

talis available. For that animal, emission measurements were made on both ears.

3. Results

Fig. 1 displays an example spectrum of DPOAEs, as measured in *Rana*. DPOAE spectra contained several DPOAE peaks, of which those at $2f_1 - f_2$ and $2f_2 - f_1$ are described in the present paper. As a function of primary tone frequency f_1 , the DPOAE level followed similar patterns in all *Rana* and *Xenopus* studied: DPOAEs were only observed within a limited frequency range, which was slightly lower for $2f_2 - f_1$ than for $2f_1 - f_2$.

Fig. 2 shows the frequency dependence of DPOAE levels for one *Rana* and one *Xenopus*. In *Rana* the DPOAE at $2f_1 - f_2$ was distinguishable from system noise and distortion (see below) when f_1 was larger than or equal to 310–1110 Hz (range across $n = 10$ frogs is indicated; the average across frogs was 565 Hz), and indistinguishable when f_1 was raised above 1910–2710 Hz (average 2377 Hz). This DP reached a maximum level of 28–55 dB SPL (average 40 dB SPL), for a frequency f_1 which was equal to 1010–1910 Hz (average 1521 Hz). In addition, a notch was observed in the response of six frogs (also visible in Fig. 2; circles) for $f_1 = 810$ –1510 Hz (average 1210 Hz). For the DP component at $2f_2 - f_1$ these values are 310–810 (average 465) Hz for the lower frequency, 1810–2610 (average 2188) Hz for the upper frequency, and a level of 31–52 (average 42) dB SPL at the optimum frequency 810–1710 (average 1376) Hz. The notch was present in five cases at $f_1 = 810$ –1210 (average 1070) Hz.

Fig. 2. DPOAE level versus primary frequency f_1 for one *R. pipiens* and one *Xenopus laevis*. Levels are shown for the DP at $2f_1 - f_2$ (*Rana*: closed circles; *Xenopus*: closed triangles) and at $2f_2 - f_1$ (*Rana*: open circles; *Xenopus*: open triangles). The ranges of system noise and distortion levels are also shown, again at $2f_1 - f_2$ (dark gray band, thin solid lines) and at $2f_2 - f_1$ (light gray band, thin dashed lines); see main text.

Fig. 3. Level of the DPOAE component at $2f_1-f_2$ versus primary frequency in four species. Each panel shows results in two ears (solid and dashed lines) along with system noise and distortion levels (gray band). (a) *Bombina orientalis*, (b) *Xenopus laevis*, (c) *Scaphiopus couchii* and (d) *Rana pipiens*. For *Bombina*, results for the two ears of the single frog are shown. For the other frogs results are shown for two frogs, one left ear each. DPOAEs were present in *Xenopus* and *Rana*, but were indistinguishable from system noise and distortion in *Bombina* and *Scaphiopus*.

In the two *Xenopus* specimens, the DPOAE levels were much lower than in *Rana*, although the f_1 frequency range was somewhat similar (see Fig. 2). In the two ears investigated, DPOAE levels at $2f_1-f_2$ rose above the system distortion when f_1 was in the frequency interval 610–2310 Hz (in one ear) and 610–2510 Hz (in the other ear). The maximum DPOAE levels were 14 and 20 dB SPL, respectively, and were observed for $f_1 = 1210$ Hz. The DP at $2f_2-f_1$ was observed in the frequency range 510–1710 Hz in one ear and 610–1910 Hz in the other ear. At $f_1 = 1010$ Hz the maximum DPOAE level was observed. This maximum was 15.0 and 18.2 dB SPL, for the two *Xenopus* studied. Pronounced notches were observed in *Xenopus* as in some *Rana* specimens. However, in *Xenopus*, for each DP (i.e. $2f_1-f_2$ and $2f_2-f_1$) two notches rather than one were observed and these were below the system distortion level. Since the DPOAE levels in *Xenopus* were close to the system distortion, we concluded that the notches were caused by destructive interference between the DPOAE and system distortion, and thus signify the presence of an OAE at the notch frequency.

In addition to DPOAE levels, Fig. 2 indicates the limitations of our recording system. System noise and distortion levels were determined with a procedure iden-

tical to the actual emission recordings, but with the microphone probe pressed against a hard surface. That is, for each set of stimulus tones, the spectral levels at $2f_1-f_2$ and $2f_2-f_1$ were stored. Often a small peak was present in the spectrum, which reflected non-linear distortion of the recording setup. Alternatively, system distortion was below the microphone noise, and the noise level at $2f_1-f_2$ and $2f_2-f_1$ was noted.

Below 400 Hz, our ability to detect emissions was limited by microphone noise. Above 400 Hz, we were limited by distortion of the measuring system in response to the stimulus tones. As a consequence, throughout the frequency range of interest, we were able to discriminate a DPOAE component whenever it was above about 5 dB SPL. The gray bands in Fig. 2 indicate the range of system noise and distortion levels obtained over subsequent experiments.

Fig. 3 displays the level of the DP at $2f_1-f_2$ versus stimulus frequency for two ears in each of the frog species studied. DPOAEs were present in all *Xenopus* and *Rana*, but none were distinguishable from system distortion and noise in *Bombina* and *Scaphiopus*.

During emission measurements, the depth of anesthesia could be estimated from the presence of movement artefacts in the microphone signal. Typically, the num-

Fig. 4. Tympanic membrane or otic skin amplitude responses versus stimulus frequency. Stimulus: free-field pure tones, 90 dB SPL. Results are shown for the same subjects illustrated in Fig. 3 with dashed and solid lines, respectively. For *Bombina* (panel a) only one ear was measured. The response to sound was up to about 0 dB re 1 mm/s in *Scaphiopus* and *Rana*, but much less in *Bombina* and *Scaphiopus*.

ber of artefacts would increase as the experiment progressed. We did not observe any dependence of emission levels on the depth of anaesthesia.

Fig. 4 displays tympanic membrane or otic skin response curves for the four species included in our study. The response of the tympanic membrane of *Rana* was typically above that of the shoulder region of this species at all frequencies. An average response curve was obtained by averaging the curves of all *Rana* subjects, i.e. for each frequency the response amplitude in absolute units was averaged across frogs. At low frequencies, the average curve rose with a slope of about 19 dB/octave. The peak membrane velocity of the average was 1.62 mm/s (4.2 dB re 1 mm/s) at 1620 Hz, the response rising up to 47.2 dB higher than that of the shoulder. The fall-off at higher frequencies was about –24 dB/octave. In *Scaphiopus*, the membrane response was clearly above the response of the shoulder from about 450 Hz. The slope of the average response rose at about 26 dB/octave to peak at 0.92 mm/s (–0.7 dB re 1 mm/s) at 1500 Hz. The average tympanic membrane response was up to 35.0 dB higher than the shoulder response. The high-frequency fall-off was around –25 dB/octave. The small peaks at 480 Hz were probably due to the table vibration at this frequency.

The tympanic membrane response of *Rana* was slightly higher than that of *Scaphiopus* and it occurred

at a higher frequency. However, the most notable difference was the much broader-band response: at 10 dB below the peak amplitude, the average bandwidth was 1660 Hz in *Rana* and 1240 Hz in *Scaphiopus*.

The response of the skin over the left otic region of *Xenopus* was found to be very low at all frequencies compared to the tympanic membrane responses of *Rana* and *Scaphiopus*. However, it varied considerably between the two *Xenopus* specimens examined. In the first specimen, the response of the otic skin was very close to that of the shoulder region at all frequencies up to around 2200 Hz. There was a peak of 0.015 mm/s (–36.2 dB re 1 mm/s) at 2910 Hz. The response of the second *Xenopus* was higher than that of the shoulder region at frequencies above 510 Hz and up to 25 dB higher than the response of the other specimen at corresponding frequencies. It showed a peak of 0.069 mm/s (–23.2 dB re 1 mm/s) at a frequency of 1350 Hz, then a slight drop followed by a secondary peak (0.058 mm/s; –24.8 dB re 1 mm/s) at 2820 Hz. The difference between the two *Xenopus* specimens might have related to differences in the position of the beads, which could only be placed in the estimated position of the tympanic disk.

The response of the otic region skin in the single *B. orientalis* included in the study was very small and peaked at 0.028 mm/s (–31.2 dB re 1 mm/s) at 360 Hz,

dropping by up to 20 dB at frequencies above 1 kHz. However, the response of the otic skin was very close to that of the shoulder region across the entire frequency range and was actually lower than the shoulder response at most frequencies.

4. Discussion

We investigated the presence of OAEs in four frog species, in order to test two hypotheses (Van Dijk et al., 1996). Before discussing our results in relation to these hypotheses, we will briefly discuss (1) the tympanic membrane laser measurements, (2) the DPOAEs in *Rana* and *Xenopus*, in relation to the frequency sensitivity ranges of the amphibian and basilar papillae, and (3) the possible effect of anesthesia on our results.

The tympanic membrane responses of both *Rana* and *Scaphiopus* showed a band-pass shape characteristic of anuran tympanic membrane velocity responses measured under free-field conditions (see e.g. Hetherington, 1992, 1994). Our results in *Xenopus* are consistent with results by Christensen-Dalsgaard et al. (1990), who demonstrated a nearly flat response of the exposed tympanic disk in this species, with a peak velocity maximum of 0.3 mm/s at 1 kHz, for airborne sound of 100 dB SPL. In addition, Hetherington and Lindquist (1999) studied the response of the lateral head region in *Bombina* and observed a similar, low-pass response to that seen in the present study. The peak in the response was lower in terms of both amplitude and frequency than that observed in the present study: around –48 dB re 1 mm/s at 200 Hz, with a second peak of similar amplitude around 900–1000 Hz, using 90 dB SPL tones.

We included the laser measurements in our study in order to determine whether sound generated in the inner ear would be able to be transmitted by the middle ear apparatus and be recorded as OAEs. Although we did not study sound transmission through the middle ear, and bearing in mind that tympanic membrane responses under free-field conditions differ from those measured under closed-field conditions in frogs (Pinder and Palmer, 1983; Vlaming et al., 1984; Aertsen et al., 1986), we assume that a significant response of the tympanic membrane or otic skin to airborne sound reflects the function of the middle ear as a sound path. In other words, we conclude from our results that sound generated in the inner ear should be measurable as OAEs in *Rana* and *Scaphiopus*. In contrast, for *Xenopus* and *Bombina* such sound would be significantly attenuated and might be undetectable.

We were able to show DPOAEs in all *Rana* and *Xenopus* studied, but in none of the *Scaphiopus* and *Bombina*. In *Rana*, emissions were observed for f_1 be-

tween (on average) 565 and 2377 Hz, while a notch in emission amplitude was present in six frogs at 1210 Hz (average). Auditory nerve fibers from the amphibian and basilar papilla have characteristic frequencies in the ranges 60–900 Hz and 1.2–1.4 kHz, respectively (Frishkopf and Goldstein, 1963; Feng et al., 1975; Christensen-Dalsgaard and Narins, 1993). Comparison of the sensitivity ranges of both papillae to the range of emission frequencies leads to the conclusion that both papillae presumably generate DPOAEs. The same conclusion was drawn by Van Dijk and Manley (2001) for *H. cinerea*. The notch in emission amplitude presumably reflects interference between amphibian and basilar papilla DPOAEs. The absence of the notch in three specimens suggests that the relative phase angles of the amphibian and basilar papilla DPOAEs may also lead to constructive interference.

For *Xenopus*, no auditory nerve recordings have been reported, but inner ear microphonics (Wever, 1985) and behavioral (Elepfandt et al., 2000) measurements indicate an auditory range from about 100 Hz to 4 kHz. The behavioral data show an elevated threshold at 1000–1300 Hz, which may indicate the functional break between the amphibian and basilar papillae. Interestingly, DPOAEs are observed in that same frequency range, which contradicts the presence of a break in that frequency range.

To summarize, in *Rana* and *Xenopus*, the DPOAEs are generated when the stimulus tones are in the auditory frequency range. In *Rana*, both the amphibian and basilar papillae contribute to DPOAE generation, while in *Xenopus* the origin of emissions remains unclear.

The absence of DPOAEs in *Bombina* is not surprising. There is no obvious route from the inner ear to the otic skin via which sound could travel in a relatively unattenuated way to the measuring microphone. Thus, even if sound was generated in the inner ear, it might not be detectable. Hetherington and Lindquist (1999) found greatest sensitivity to airborne sound for stimuli presented to the area over the lungs. Possibly, sounds generated in the inner ear can be transmitted outward via the same route and could be detected with an emission probe placed over the lung area.

The absence of DPOAEs in *Scaphiopus* is more surprising, since *Scaphiopus* has a middle ear which we expected to transmit sound from the inner ear.

We did not observe any dependence of OAEs on depth of anesthesia in either *Rana pipiens* or *Xenopus laevis*. This is in apparent contrast to findings by Van Dijk and Manley (2001), who describe that the disappearance of movement artefacts in the microphone signal correlates with the disappearance of emissions. Presumably, the difference between our and their results relate to the anesthesia used: Van Dijk and Manley (2001) used MS222, while we used pentobarbital. Since

DPOAE levels in *Rana* and *Xenopus* do not depend on depth of anesthesia in our study, the same is probably true for *Bombina orientalis* and *Scaphiopus couchii*. In other words, the absence of DPOAEs in *B. orientalis* and *S. couchii* probably cannot be explained by anesthesia effects.

Our motivation for the present study was to test two hypotheses (Van Dijk et al., 1996) on the absence of OAEs in some frog species. OAEs have been recorded from the frog genera *Rana* (Palmer and Wilson, 1982; Van Dijk et al., 1989), *Hyla* (Van Dijk et al., 1996; Van Dijk and Manley, 2001), and *Leptodactylus* (Van Dijk et al., 1996), but SOAEs were absent in *Bombina* and *Xenopus* (Van Dijk et al., 1996). The first hypothesis was that the absence of OAEs is due to the non-tympanic ears of *Xenopus* and *Bombina*: if sound from the inner ear cannot be radiated out via a tympanic membrane, OAEs cannot be recorded. We confirmed the responsiveness of the tympanic membranes in *Rana* and *Scaphiopus* with laser measurements. The other species lack differentiated, external tympanic membranes, and the otic skin has a much reduced response to airborne sound.

The presence of DPOAEs in *Xenopus* shows that a tympanic membrane is not required for measurable OAEs. Thus, the first hypothesis of Van Dijk et al. (1996) is rejected. In addition, the absence of DPOAE in *Scaphiopus* shows that a functional tympanic membrane does not imply measurable OAEs.

The second hypothesis put forward by Van Dijk et al. (1996) relates to structural properties of the amphibian papilla. The amphibian papilla has a tonotopic organization, with the high frequencies represented on an extension caudal to a tectorial curtain (Lewis, 1984). The caudal extension varies in length across frog species (Lewis, 1981; see Table 1). In *Bombina*, the amphibian papilla stops at the tectorial curtain, while in *Xenopus* and *Scaphiopus* it extends beyond. A fully developed caudal extension is present in *Rana*, where the total length caudal to the curtain is approximately twice that of *Xenopus* and *Scaphiopus*. Van Dijk et al. (1996) hypothesized that emissions may be attenuated or abolished if the caudal extension is absent or reduced in size. If this were true, our study might only be expected to show OAEs in *Rana*. Thus, the presence of OAEs in *Xenopus* demands that we reject the second hypothesis of Van Dijk et al. (1996) as well.

Although the presence of DPOAEs is not related to structural characteristics, as predicted by both hypotheses, a relation to hearing sensitivity exists. Wever (1985) measured the auditory sensitivity in a wide variety of frog species by determining the sound level required to generate a 0.1 μ V inner ear potential. Although not a measure of hearing threshold in the usual sense, these measurements provide a measure of

hearing sensitivity. In *Rana*, the 0.1 μ V threshold is reached for stimuli as low as 32 dB SPL. *Xenopus* shows an even greater sensitivity to airborne sound, with a threshold at 27 dB SPL. *Scaphiopus* and *Bombina* are less sensitive to airborne sound, with thresholds at 56 and 64 dB SPL, respectively. Auditory nerve fiber thresholds are 20 dB SPL in *Rana* (Christensen-Dalsgaard and Narins, 1993), 40 dB in *Scaphiopus* (Capranica and Moffat, 1975) and 65 dB SPL in *Bombina* (Hillery and Narins, 1987). Thus neural and inner ear potentials indicate that both *Rana* and *Xenopus* are relatively sensitive to airborne sound, while in *Scaphiopus* and *Bombina* sensitivity is reduced. This correlates closely with our result that *Rana* and *Xenopus* have DPOAE, while *Scaphiopus* and *Bombina* do not.

It is currently unclear why *Rana* and *Xenopus* are more sensitive to sound than *Scaphiopus* and *Bombina*. The presence of OAEs is supposed to reflect a mechanism in the inner ear which enhances auditory sensitivity. Thus, it is tempting to assume that the absence of emissions in *Bombina* and *Scaphiopus* reflects the absence of this mechanism, which in turn results in reduced auditory sensitivity.

Two additional explanations might account for the absence of measurable DPOAEs in *Scaphiopus* and *Bombina*. First, although we have considered the responses of the tympanic membrane or otic skin, we cannot exclude the possibility that other aspects of middle ear structure might attenuate the transmission of DPOAEs. Under the skin and fat of the otic region in *Xenopus* is a cartilaginous tympanic disk, which is coupled to the inner ear by means of the stapes (Wever, 1985). Although a tympanic middle ear in the conventional sense is absent, the conducting apparatus remains in this modified form. *Bombina*, by contrast, lacks these structures and therefore possesses no specialized means by which otic skin vibrations may be coupled to the auditory end-organs (Wever, 1985). This could potentially account for our failure to record DPOAEs from this species. However, it is more difficult to argue that the tympanic middle ear of *Scaphiopus*, although apparently reduced in comparison to that of *Rana* (personal observation), is incapable of transmitting emissions.

Second, in *Scaphiopus* the hair cell stereocilia bundles in the basilar papilla are oriented randomly. By contrast, in *Xenopus* and *Rana*, hair bundles are aligned (Lewis, 1978). If DPOAEs are caused by hair bundle movements (Martin and Hudspeth, 1999), each individual bundle is expected to oscillate in the orientation direction. In *Scaphiopus*, the random orientation would be expected to prevent synergistic action of hair cells for emission generation, while in *Xenopus* and *Rana* individual hair bundle contributions may summate.

In conclusion, we demonstrated the presence of DPOAEs in *Rana* and *Xenopus*, but not in *Bombina*

and *Scaphiopus*. Thus, a fully developed caudal extension of the amphibian papilla and a tympanic membrane are neither necessary nor sufficient for generation of measurable DPOAEs. The presence of DPOAEs in *Rana* and *Scaphiopus* correlates with the high auditory sensitivity in these species, as opposed to a lower sensitivity in *Bombina* and *Scaphiopus*.

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