Distortion product otoacoustic emissions provide clues to hearing mechanisms in the frog ear

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2 $f_1$-$f_2$ and 2 $f_2$-$f_1$ distortion product otoacoustic emissions (DPOAEs) were recorded from both ears of male and female *Rana pipiens* and *Rana catesbeiana*. The input-output (I/O) curves obtained from the amphibian papilla (AP) of both frog species are analogous to I/O curves recorded from mammals suggesting that, similarly to the mammalian cochlea, there may be an amplification process present in the frog AP. DPOAE level dependence on L$_1$-L$_2$ is different from that in mammals and consistent with intermodulation distortion expectations. Therefore, if a mechanical structure in the frog inner ear is functioning analogously to the mammalian basilar membrane, it must be more broadly tuned. DPOAE audiograms were obtained for primary frequencies spanning the animals’ hearing range and selected stimulus levels. The results confirm that DPOAEs are produced in both papillae, with *R. catesbeiana* producing stronger emissions than *R. p. pipiens*. Consistent with previously reported sexual dimorphism in the mammalian and anuran auditory systems, females of both species produce stronger emissions than males. Moreover, it appears that 2 $f_1$-$f_2$ in the frog is generated primarily at the DPOAE frequency place, while 2 $f_2$-$f_1$ is generated primarily at a frequency place around the primaries. Regardless of generation place, both emissions within the AP may be subject to the same filtering mechanism, possibly the tectorial membrane. © 2004 Acoustical Society of America. [DOI: 10.1121/1.1811571]

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I. INTRODUCTION

A. Background—definitions

Distortion product otoacoustic emissions (DPOAEs) arise when the ear is stimulated acoustically by two sinusoidal signals (primaries) with appropriately chosen frequencies ($f_1$, $f_2$) and stimulus levels (L$_1$, L$_2$) (e.g., Kemp, 1979; Probst et al., 1991). In human subjects, otoacoustic emissions (OAEs) in general are often used for clinical diagnostic screening of frequency-dependent cochlear function. DPOAEs may be used for early screening of outer hair cell (OHC) damage (e.g., Lonsbury-Martin et al., 1993; Stover et al., 1996; Wagner and Plinkert, 1999), for monitoring inner ear function after exposure to ototoxic drugs and/or noise (e.g., Brown et al., 1989; Emmerich et al., 2000), for diagnosis of middle ear damage (e.g., Owens et al., 1992, 1993; Zhang and Abbas, 1997), and may also be used for assessing OHC maturation in premature babies (e.g., Abdala, 2000). In addition, OAEs have provided noninvasive means of examining inner ear mechanisms in mammals (e.g., Maison et al., 1997; Shera and Guinan, 2003) and other vertebrates (e.g., Rosowski et al., 1984), as well as in insects (e.g., Kössl and Boyan, 1998). The present study uses DPOAE recordings from two frog species to examine hearing mechanisms in the frog ear.

The vast majority of physiological and psychophysical studies involving DPOAEs concentrate on the 2 $f_1$-$f_2$ distortion product, principally because it usually represents the strongest DPOAE and therefore the easiest one to measure. OAEs, including DPOAEs, originate in the inner ear and, in most mammals, have been linked to outer hair cell (OHC) motility and receptor potential (Probst et al., 1991; Robles and Ruggero, 2001). More specifically, the shape of mammalian DPOAE input/output (I/O) curves (i.e., compressive growth at low stimulus levels, although not necessarily as compressive as in the case of basilar membrane and hair cell I/O curves) provides further evidence that may link DPOAE generation to the cochlear amplifier, a process hypothesized to account for the observed increased sensitivity of the mammalian ear at low stimulus levels and for the mammalian ear’s compressive and saturating response as stimulus levels increase from low to intermediate. Zheng et al. (2000) showed that prestin is the motor protein related to OHC motility, while Liberman et al. (2002) demonstrated a direct coupling between electrically stimulated OHC motility and cochlear amplification. In general, DPOAE generation in mammals has also been associated with the overlap of the
primary tones’ basilar membrane (BM) disturbances.

DPOAEs have been recorded from the ears of all terrestrial vertebrate classes, including most frog species tested. The presence of OAEs in general and DPOAEs in particular in the frog raises numerous questions with regard to emission origin, especially due to the fact that the frog inner ear has neither a BM (Capranica, 1976) nor morphologically distinct outer and inner hair cells (Lewis, 1977; Lewis et al., 1982). Based on observations that direct current injection may influence both the frequencies and amplitudes of OAEs (Wit et al., 1989), Probst et al. (1991) suggested that frog OAEs may be related to the electrical tuning of hair cells. Others (e.g., Van Dijk et al., 2002) have suggested that OAEs in frogs may be linked to spontaneous hair bundle movement, which may be analogous to the previously mentioned OHC motility in mammals and has been shown to occur in the bullfrog sacculus (Martin and Hudspeth, 1999). Hair bundle movement has been observed in vitro on several nonmammalian vestibular organs (review in Hudspeth, 1997) and the hearing organ of turtles (Crawford and Fettiplace, 1985), and in vivo on the bobtail lizard (Manley et al., 2001). Recently, hair bundle motility was also observed in rats (Kennedy et al., 2003).

The frog ear responds to airborne sound via two separate hearing organs, the amphibian papilla (AP) and the basilar papilla (BP). The AP nerve fibers are tuned to low and middle frequencies while the BP fibers are tuned to high frequencies (Feng et al., 1975). More specifically, in R. p. pipiens the AP responds best to frequencies approximately within the range 100–1250 Hz, while the BP responds best to frequencies approximately within the range 1250–2400 Hz, with a “break” in the frequency response of the two papillae at approximately 1250 Hz (Ronken, 1991). In R. catesbeiana, the AP range is approximately 100–1100 Hz and the BP range is approximately 1100–1700 Hz, with the frequency response border at approximately 1100 Hz (Feng et al., 1975; Lewis et al., 1982). The present study reveals similarities in the behavior of DPOAEs originating in the frog AP and the mammalian cochlea, supporting analogies between these two organs. Several such analogies and distinctions have already been pointed out (Lewis and Narins, 1999), guiding the present study.

B. Exploration of the stimulus parameter space

The results of parametric studies (e.g., Whitehead et al., 1995a, b; Knight and Kemp, 2000; Kummer et al., 2000; Mills, 2002; Schneider et al., 2003) have revealed a complex interrelationship between DPOAE levels and the four-dimensional primary-tone parameter space and have guided mammalian models of DPOAE generation (e.g., Shera and Guinan, 1999; Talmadge et al., 1998, 1999; Tubis et al., 2000; Knight and Kemp, 2000; Fahey et al., 2000; Lukashkin et al., 2002; Mills, 2002). The precise relationship may not be the same for all mammals, but it exhibits several distinguishing trends. As an example, a generally accepted protocol leading to optimal DPOAE generation within the frequency ranges of interest in clinical DPOAE measurements includes the following:

(a) Frequency range: For the DPOAE audiograms, the primary-tone frequencies were selected based on the tuning characteristics of the R. p. pipiens and R. catesbeiana ears, spanning the frequency range of each papilla (AP and BP) and at frequency steps approximately equal to or less than 10% of $f_1$.

(b) $L_1 \leq 65$ dB SPL. For most mammals, DPOAE I/O curves show saturation at stimulus levels around 60–70 dB SPL (Probst et al., 1991), followed by a DPOAE level decrease.

(c) $5 \leq L_1 - L_2 \leq 10$ dB, for $f_2/f_1 \geq 1.2$ (e.g., Whitehead et al., 1995a, b; Kummer et al., 2000; Fahey et al., 2000). As primary-tone levels become higher and/or frequency ratio becomes smaller, this level difference becomes increasingly unnecessary for the maximization of DPOAE levels.

In frog DPOAE studies, the selection of primary tone parameters has in general been less systematic. For example, Van Dijk et al. (2002) used $f_2/f_1 = 1.1$ and $L_1 = L_2 = 90$ dB SPL. Van Dijk and Manley (2001) used $1.05 \leq f_2/f_1 \leq 1.5$ and $37 \leq L_1 = L_2 \leq 85$ dB SPL, but only reported the relationship between DPOAE audiograms and $f_2/f_1$. In the absence of both a full parametric study and a theoretical model of DPOAE generation customized to the amphibian ear, parameter choices have been based on mammalian theoretical grounds, on DPOAE data from studies on other nonmammals, or on a trial-and-error basis.

The present work provides a more systematic exploration of the primary-tone parameter space in frogs. DPOAE amplitudes were recorded from two ranid frog species in a series of experiments that examine the dependence of DPOAE amplitude on the absolute frequencies and on the absolute and relative levels of the primaries. In addition, we examined the effects of frog species and sex on DPOAE levels, controlling for possible effects of the degree of anesthesia.

II. MATERIALS AND METHODS

A. Stimulus parameter space

The dependence of frog DPOAE levels on primary-tone frequency ratio (explored extensively in mammals, e.g., Knight and Kemp, 2000; Schneider et al., 2003) was not addressed in the present study and the primary-tone frequency ratio was set at $f_2/f_1 = 1.15$ for all experiments. In the absence to date of a parametric study examining the relationship between DPOAEs and $f_2/f_1$ in the frog, this value was chosen based on the previously mentioned recommendation ($f_2/f_1 \geq 1.15$) and to facilitate comparison with previous frog research (Van Dijk et al., 2002: $f_2/f_1 = 1.1$). More specifically, the study addresses the following primary-tone parameters:

(a) $1.2 \leq f_2/f_1 \leq 1.25$. Shera and Guinan (1999), Faulstich and Kössl (2000), and others have argued that this value must be at least $f_2/f_1 = 1.15$, to avoid phase complications. In human subjects, $f_2/f_1 = 1.22$ results in the strongest $2f_1-f_2$ emissions (Probst et al., 1991).
DPOAE amplitudes are thought to be independent of stimulus levels. Highest DPOAE levels and lowest DPOAE thresholds have in general been measured for primaries within the frequency ranges associated with the most sensitive hearing regions of the species examined (Probst et al., 1991). Additionally, there is a close relationship between auditory threshold and EOAE levels (Wagner and Plinkert, 1999). Therefore, for the DPOAE I/O curves, \( f_1 \) was selected so that all frequencies of interest (\( f_1, f_2, 2f_1-f_2, 2f_2-f_1 \)) fell near the center of each papilla’s frequency range.

(c) \textit{Primary-tone relative levels} DPOAE audiograms were also obtained for unequal levels of the primaries (0 = \(|L_1-L_2| \leq 10\) dB) from \( R. p. pипiens \) at \( 300 \leq f_1 \leq 1600 \). The frequency range examined was selected based on the tuning characteristics of the \( R. p. pипiens \) AP.

**B. Subjects and procedure**

DPOAEs were recorded from both ears of

(a) Ten northern leopard frogs (\( R. p. pипiens \)), five males (body mass 25.04–54.31 g, snout-vent length 6.08–8.08 cm, cranial width 2.09–3.05 cm, tympanic membrane diameter 0.41–0.53 cm) and five females (body mass 26.59–56.02 g, snout-vent length 6.94–7.61 cm, cranial width 2.32–2.57 cm, tympanic membrane diameter 0.41–0.52 cm) and

(b) Ten bullfrogs (\( R. catesbeiana \)), five males (body mass 267–370 g, snout-vent length 15.29–17.41 cm, cranial width 5.24–5.95 cm, tympanic membrane diameter 1.63–2.16 cm) and five females (body mass 300–435 g, snout-vent length 14.76–17.55 cm, cranial width 4.82–6.02 cm, tympanic membrane diameter 1.21–1.48 cm).

DPOAE amplitudes are thought to be independent of stimulation side (i.e., left versus right ears) and hearing loss in one ear of a subject does not affect emission levels in the other (Probst et al., 1991; Kastanioudakis et al., 2003). Therefore, each ear tested in our study was considered a separate data point.

The same subjects participated in all experiments, performed in a single session per subject, lasting 4–9 h. Animals were anesthetized with an intramuscular injection of pentobarbital sodium solution (Nembutal, Abbott Laboratories, 50 mg/ml: \( \sim 0.9–1.0 \) \( \mu \)g body mass) and were covered with wet gauze that was regularly moistened with water to facilitate cutaneous respiration. Measurements began between approximately 1.5 and 3 h after Nembutal injection, with the animals deeply anesthetized (no toe-pinch or eye reflex and no acoustic artifacts—i.e., breathing/swallowing noise—in the recorded signal). Some of the DPOAE measurements on \( R. p. pипiens \) were repeated 5–6 h later under light anesthesia (all reflexes had returned and there were acoustic artifacts present in the microphone signal) to control for possible effects of the degree of anesthesia on emission levels.

**FIG. 1.** DPOAE I/O curves (\( 2f_1-f_2 \) from the AP (\( f_1 = 600 \) Hz) of male (\( \bullet \)) and female (\( \square \)) \( R. catesbeiana \). Average DPOAE levels and standard errors (ten ears each sex; both ears of five males and five females). Although there are level differences between male and female DPOAEs, the overall shape of the I/O curves is similar regardless of subjects’ sex.

**C. Equipment and signal analysis parameters**

Measurements were performed in a single-wall sound-attenuating chamber with the subjects placed on a vibration isolation table (Newport VH Isostation). Emissions were recorded with a probe assembly (ER-10C, Etymotic Research, Elk Grove, IL) that includes a sensitive microphone connected to a preamplifier (amplification: 20 dB) and two miniature speakers. The ER-10C probe is designed and calibrated for use with human subjects. Since the frog species examined have no ear canals, the probe ear-tip was placed inside a plastic tube, which was coupled to the frog ear by placing its open end adjacent to the skin around the tympanic membrane. The small gap between the plastic tube and the frog’s head was sealed using high vacuum grease, a silicone lubricant. The integrity of the acoustic seal was tested using a sweep tone (WG1 waveform generator, Tucker-Davis Technologies, Alachua, FL) to ensure that sound level loss at 250 Hz due to diffraction was \( \leq 5 \) dB, relative to the level measured at 1000 Hz. Because of the large differences in tympanic membrane diameter between \( R. p. pипiens \) and \( R. catesbeiana \) and between male and female \( R. catesbeiana \), three different “ear canal” tubes were used: (1) \( R. p. pипiens \): diameter \( \leq 0.95 \) cm, length \( \leq 1.20 \) cm, volume \( \leq 0.68 \) cm\(^3\); (2) male \( R. catesbeiana \): diameter \( \leq 2.20 \) cm, length \( \leq 0.55 \) cm, volume \( \leq 2.10 \) cm\(^3\), and (3) female \( R. catesbeiana \): diameter \( \leq 1.25 \) cm, length \( \leq 1.40 \) cm, volume \( \leq 1.72 \) cm\(^3\).

Primary frequencies (\( f_1 \)) were selected within the range 240–3000 Hz\(^5\) and stimuli were generated using a Real Time Processor (TDT-RP2), controlled by software written in Matlab (version 12, MathWorks, Inc., Natick, MA) and RPdvgs (version 5.0, TDT, Alachua, FL). The stimuli were fed to the two miniature speakers on the probe via two attenuators (TDT-PA4), used for manual control of the signal levels. The...
probe’s microphone signal was fed to a frequency analyzer (SRS-SR770 FFT Network Analyzer, Stanford Research Systems, Sunnyvale, CA). The bandwidth of the Fourier analysis changed as a function of $f_1$. For each stimulus tone-pair, the DPOAE level reported was the maximum amplitude of five adjacent analysis bands centered at the band containing the DPOAE frequency. The noise level was calculated by averaging the levels of 18 analysis bands surrounding the five bands used to determine the DPOAE level. These DPOAE- and noise-level calculation methods minimized the influence of spectral leakage and gave noise levels comparable to system distortion ($\approx 83$ dB below the primaries for $L_1 = L_2 \geq 70$ dB SPL and $\approx 12$ dB SPL for $L_1 = L_2 < 70$ dB SPL), but may have overestimated DPOAE levels that were near the calculated noise floor. Therefore, any emission levels in the results that are $< 3$ dB above the estimated noise-floor may be considered indistinguishable from noise. All levels reported have been corrected to compensate for the frequency response of the ER-10C microphone.

III. RESULTS

A. Experiment 1: DPOAE I/O curves

DPOAE I/O curves at $2f_1 - f_2$ and $2f_2 - f_1$ were obtained for $f_2/f_1 = 1.15$ and $35 \leq L_1 = L_2 \leq 85$ dB SPL (in 2.5-dB steps) with $f_1 = 800$ Hz (AP) and 1800 Hz (BP) for $R. p. picipiens$ and $f_1 = 600$ Hz (AP) and 1500 Hz (BP) for $R. catesbeiana$. Although the absolute DPOAE amplitudes were different for male and female subjects, the overall shapes of DPOAE I/O curves within each species were similar between the two sexes (Fig. 1), so data obtained from male and female animals were averaged. Mean levels and standard errors for each distortion product from each papilla and for both species are displayed in Fig. 2.

In Fig. 2(a), the $2f_1 - f_2$ DPOAE I/O curves obtained from the AP of both $R. p. picipiens$ and $R. catesbeiana$ display non-monotonic growth. For low primary levels ($< 60$ dB SPL), the DPOAE level growth rate is $< 1$ dB/dB (compressive), saturating at primary levels $\approx 60$ dB SPL, turning negative, and reaching a notch at primary levels $\approx 70$ dB SPL ($\approx 67$ dB SPL for $R. catesbeiana$; $\approx 72$ dB SPL for $R. p.$.

FIG. 2. DPOAE I/O curves for $f_2/f_1 = 1.15$ from $R. p. picipiens$ (–– $f_1 = 800$ Hz, 1800 Hz) and $R. catesbeiana$ (–– $f_1 = 600$ Hz, 1500 Hz). Average DPOAE levels and standard errors (20 ears each species: both ears of five males and five females). (a) and (b) $2f_1 - f_2$; (c) and (d) $2f_2 - f_1$; (a) and (c) AP; (b) and (d) BP.
While the deviation from linear growth in the $\gamma$-curve is observed at $L_1$, minima in DPOAE amplitude were found did not change frequency. The frequencies for which respective maxima and periodic variation in DPOAE amplitude as a function of frequency from each papilla. Similar responses were observed at other frequencies (Fig. 5). Again, although the absolute DPOAE amplitudes were different for male and female subjects, there were no significant sex differences in the shape/slopes of the curves describing the relationship between $L_1$-L$_2$ and DPOAE levels. Therefore, data obtained from male and female animals were averaged.

Independent of species, sex, and DPOAE (2 $f_1$-$f_2$ or 2 $f_2$-$f_1$), strongest emissions were recorded for $|L_1$-$L_2|$ = 0 or 2.5 dB. As $|L_1$-$L_2|$ increased, DPOAE amplitudes gradually dropped. Figure 5 displays the same results in the form of DPOAE audiograms, showing maximum DPOAE levels for $L_1$-$L_2$ = 0 for most frequencies tested and both 2 $f_1$-$f_2$ and 2 $f_2$-$f_1$.

C. Experiment 3: DPOAE audiograms

1. AP versus BP; 2 $f_1$-$f_2$ and 2 $f_2$-$f_1$

DPOAE audiograms were obtained from both ears of all subjects for $240 < f_1 < 3000$ Hz at $\sim 0.1 f_1$ steps, with $L_1$ = $L_2$ = 60 dB SPL, and $f_2/f_1$ = 1.15. Figure 6 (R. p. pipiens) and Fig. 7 (R. catesbeiana) display the levels of 2 $f_1$-$f_2$ and 2 $f_2$-$f_1$ DPOAEs as a function of (a) stimulus frequency $f_1$, (b) stimulus frequency $f_2$, and (c) distortion product (DP) frequency. Each plot includes a dashed vertical line that indicates the approximate frequency value separating the frequency ranges of the two papillae. For both species, the frequency separation between the two papillae is based on neural tuning curve characteristics (R. p. pipiens: 1250 Hz, Ronken, 1991; R. catesbeiana: 1100 Hz, Feng et al., 1975). DPOAE data from both sexes were averaged. The error bars in Figure 9 and 10 are an indication of the variability in the male and female date.

In both figures, two distinct frequency regions can be identified where relative maximum emission amplitudes are found. These frequency regions correspond roughly to the

\[ a \cdot b \cdot c \cdot d \cdot e \cdot f \cdot g \cdot h \cdot i \cdot j \]
frequency tuning of the AP (lower frequency region) and the BP (higher frequency region) of each species, respectively. For the primary level tested (60 dB SPL), the emission levels recorded from the AP were higher than those recorded from the BP.

Figure 6 indicates that, for \( R. \ p. \ pipiens \), alignment of the \( 2f_1-f_2 \) DPOAE audiogram dip and the break in the frequency coverage between the two papillae (1250 Hz; Ronken, 1991) is best when \( 2f_1-f_2 \) amplitude is plotted as a function of distortion product frequency [Fig. 6(c)]. For the \( 2f_2-f_1 \) DPOAE, on the other hand, closest alignment occurs when \( 2f_2-f_1 \) amplitude is plotted as a function of \( f_2 \) [Fig. 6(b)]. Near alignment is also observed when this distortion product is plotted as a function of \( f_1 \) [Fig. 6(a)]. Similar results were obtained for \( R. \ catesbeiana \) (Fig. 7). All values reported are averages.

To control for possible effects of the degree of anesthesia on DPOAE amplitudes, the experiment was repeated 5–6 h later as the anesthesia was wearing off (eye and toe-pinch reflexes accompanied by acoustic artifacts—i.e., breathing/swallowing noise—in the recorded signal). “Light” anesthesia measurements were made on all \( R. \ p. \ pipiens \) for \( 300<f_1<1600 \) Hz, \( L_1=L_2=60 \) dB SPL, and \( f_2/f_1=1.15 \). The mean results are displayed in Fig. 8 and indicate that, for the anesthetic agent used and the frequencies and levels tested, degree of anesthesia did not affect DPOAE amplitudes.

2. Female versus male; \( R. \ p. \ pipiens \) and \( R. \ catesbeiana \)

Separate analyses of DPOAE data from male and female \( R. \ p. \ pipiens \) and \( R. \ catesbeiana \) revealed clear sex differences in DPOAE levels. Figures 9 and 10 compare male and female DPOAE audiograms, demonstrating that, in both species, over most of the frequency range tested, and for the levels of primary tones used, female subjects produce higher emission levels than male subjects. This is more pronounced in the BP.

With the exception of emissions from the high-frequency end of the BP, \( R. \ catesbeiana \) demonstrate larger emission level differences between sexes and, as also indicated in Fig. 2, larger emission levels in general.
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are ≪1 dB/db. For intermediate stimulus levels (L1 = L2
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ing in a decrease in DPOAE amplitude (i.e., a notch). For
higher stimulus levels (L1 = L2 > 70 dB SPL) the slope in-
creases again, often to a value >1 dB/db (e.g., Probst
1991). In mammals, it has been shown that DPOAE levels in
general (e.g., Knight and Kemp, 2000) and the exact shape
of the DPOAE I/O curves in particular (e.g., Mills, 2002)
depend on the distortion product under study, and on the
relative levels (L1−L2) and frequencies (f2/f1) of the prima-
ries. In our DPOAE I/O experiment, L1−L2 and f2/f1 were
not varied. The AP data, however, do exhibit differences in
I/O curve shapes between distortion products [Fig. 2(a) ver-
sus 2(c)], with only the I/O curve for the 2f1−f2 DPOAE
exhibiting a clear notch. Our results are in general agreement
with the DPOAE I/O curve slopes measured by Meenderink
and Van dijk (2004) on R. p. pipiens, although, in their re-
results, both the low- and high-level I/O slopes obtained from
the BP appear relatively steep.

Until recently, the main hypothesis (e.g., in Probst et al.,
1991) introduced to explain the DPOAE I/O curve shape
(especially the observed notches) in mammals assumes that
there is interference between 2 DPOAE components:

(a) a level dependent, nonlinear component, which domi-
nates the recorded DPOAEs when the stimulus levels are
low but saturates for higher stimulus levels and
(b) a monotonic component that, as the stimulus levels in-

IV. DISCUSSION

A. DPOAE I/O curves provide evidence of an AP
amplifier

The 2f1−f2 I/O curves obtained from the AP of both
frog species tested [Figs. 1 and 2(a)] have a salient charac-
teristic in common with the DPOAE I/O curves obtained
from mammals: nonlinear, non-monotonic growth. The gen-
eral shape of I/O curves recorded in mammals is described in
a manner strikingly similar to the one used to describe the
2f1−f2 DPOAE I/O AP curves obtained in our study [Fig.
2(a)] and, with the absence of a clear notch, to the one de-
scribing the 2f2−f1 DPOAE I/O AP curves [Fig. 2(c)]. The
slope of mammalian DPOAE I/O curves depends on the
stimulus levels used. For primary levels <60 dB SPL slopes
are ≪1 dB/db. For intermediate stimulus levels (L1 = L2
≈ 60–70 dB SPL) the growth saturates, sometimes even re-
ing in a decrease in DPOAE amplitude (i.e., a notch). For
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nates the recorded DPOAEs when the stimulus levels are
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(b) a monotonic component that, as the stimulus levels in-
crease and the level-dependent component saturates, becomes dominant in the recorded DPOAEs.

The precise nature of these two components and their place of origin are subjects of ongoing research.

Lukashkin et al. (2002) proposed an alternative hypothesis with subtle but important differences to the previous one. Based on the observation that cochlear function impairment results in an upward level shift of the DPOAE I/O curve notch as opposed to the downward level shift predicted by the earlier hypothesis, they concluded that DPOAE I/O curve shapes can be accounted for by a single source: a nonlinear amplifier with saturating I/O characteristics. A similar hypothesis was introduced by Mom et al. (2001) based on their observation that DPOAEs measured during complete cochlear ischemia are highly vulnerable to mild auditory fatigue induced prior to ischemia. Their results indicate that there is no “passive” component in the response of the mammalian cochlea and support the single-component model. Whitehead (1998) and clinical data reviewed in Martin (2002) also support such a model.

The common thread in all explanations is the hypothesis of a saturating nonlinear inner ear amplifier, i.e., cochlear amplifier. The DPOAE I/O curves in Fig. 2a of the present study, along with the fact that SOAEs have been recorded from the frog AP, are consistent with the inner ear amplifier hypothesis, despite the fact that the frog ear lacks OHCs, believed to be at the center of the mammalian ear’s amplification process. Amplification and OAEs, in the AP of the frog, may be linked to spontaneous movement of the hair cell bundle, which has been shown to occur in the frog sacculus (Martin and Hudspeth, 1999), while there is indirect evidence that it may also occur in the mammalian ear (Kennedy et al., 2003). In other words, hair cell bundle movement in the frog AP may be analogous to OHC and/or hair bundle motility in the mammalian ear.

For primary frequencies in the BP, DPOAE levels were much lower than those recorded from the AP. The portions of the DPOAE I/O curves that are distinguishable from the noise-floor appear monotonic, implying that, in contrast to the AP, an amplification mechanism may not be present in the frog BP. This potential difference is consistent with the absence of SOAEs in the frog BP (Van Dijk et al., 1989) and the differential physiological vulnerability of frog DPOAEs.

FIG. 7. Average levels of the $2f_1 - f_2$ (—) and $2f_2 - f_1$ (——) DPOAEs from R. catesbeiana (20 ears: both ears of five males and five females) for $f_2/f_1 = 1.15$, $L_1 = L_2 = 60$ dB SPL ($\pm 2.5$ dB), and $240 < f_1 < 3000$ Hz as a function of (a) $f_1$, (b) $f_2$, and (c) DP frequency. The alignment between the dip in the audiograms and the frequency coverage border between the AP and the BP follows the same pattern as in the R. p. pipiens data (Fig. 6).

FIG. 8. DPOAE audiograms from R. p. pipiens (average DPOAE levels from 20 ears: both ears of five males and five females) for $f_2/f_1 = 1.15$, $L_1 = L_2 = 60$ dB SPL, and $300 < f_1 < 1600$ Hz, recorded from the same animals while heavily and lightly anesthetized. No systematic effect of the degree of anesthesia on DPOAE levels was observed. (a) $2f_1 - f_2$; (b) $2f_2 - f_1$.
and may echo several physiological differences between the amphibian and basilar papilla. In the bullfrog ear, for example, the AP has approximately 15 times as many hair cells and is innervated by approximately three times as many afferent nerve fibers as the BP (Geisler et al., 1964; Lewis et al., 1985). At the same time, efferent nerve fibers innervate the AP but not the BP (Robbins et al., 1967). The possible relationship between these physiological differences and the observed difference in DPOAE I/O curves, assumed to reflect amplification-process differences, remains to be examined. As a general observation, the fact that only the AP is tonotopically organized (Lewis et al., 1982; Simmons et al., 1992) is consistent with a greater relevance of an amplification mechanism that would increase this papilla’s tuning sharpness. In contrast, the BP responds as a band-pass filter with a single characteristic frequency (e.g., Van Dijk and Manley, 2001), rendering the development of an amplification and tuning-sharpening mechanism less essential.

In both R. p. pipiens and R. catesbeiana, the peak of the compressive-with-saturation portion of the DPOAE I/O curves occurs for \( L_1 = L_2 \sim 60 \) dB SPL. Since in mammals the amplified or “active” portion of the I/O curves is presumed to be dominant only at low stimulus levels, DPOAE audiograms (experiment 3) were obtained for \( L_1 \) and \( L_2 \) centered at \( \sim 60 \) dB SPL. The relative levels of the primaries \( (L_1-L_2) \) corresponding to the highest DPOAE levels were determined in a separate experiment, discussed below.

**B. DPOAE amplitude dependence on the relative levels of primaries**

In mammals, the amplitude of each distortion product is influenced most by level changes in its neighboring (in frequency) primary tone, consistent with intermodulation distortion expectations. However, and contrary to intermodulation distortion expectations, the highest DPOAE levels are obtained for unequal levels of the primaries (i.e., \( L_1 > L_2 \); Kummer et al., 2000). This asymmetry in primary tone levels has been linked to the asymmetry of basilar membrane disturbance envelopes and is less critical at low frequencies, high primary stimulus levels, and low frequency ratios of the primaries. Our findings in the frog are more consistent with intermodulation distortion expectations alone, with highest emission levels recorded for equal levels of the primaries (Figs. 4 and 5). The difference between mammalian and frog data regarding the effect of \( L_1-L_2 \) on DPOAE levels suggests that, if a filtering mechanism analogous to the mammalian BM is present in the frog (i.e., tectorial membrane), it must be more broadly tuned. This notion is supported by the
shape of frequency threshold curves (i.e., tuning curves) recorded from frog VIIIth nerve auditory fibers [e.g., R. cates-beiana in Feng et al. (1975); Eleutherodactylus coqui and Bombina orientalis in Narins and Hillery (1983); R. p. pipiens in Ronken (1991)]. In both mammals and frogs, tuning curves display an asymmetry, with relatively shallow low-frequency limbs and high-frequency abrupt cutoffs, which is stronger for high-frequency fibers. However, neural tuning curves recorded from mammals (e.g., Robles and Ruggero, 2001) exhibit narrower tips than those recorded from frogs. In mammals, the above noted asymmetry, along with several other properties of auditory nerve responses including the L1, L2 DPOAE asymmetry, is thought to reflect corresponding features of BM vibration (Robles and Ruggero, 2001). Our results (Figs. 4 and 5), along with the difference between mammalian and frog neural tuning curves, suggest that, although the AP and BP of the frog ear lack a BM, some other mechanical structure (possibly the tectorial membrane) may in some respects function analogously to the mammalian BM, while more broadly tuned. This observation is in agreement with phase data from auditory nerve fiber measurements in the frog (Hillery and Narins, 1987), pointing to the tectorial membrane–receptor interface as a possible candidate for the mechanical generation of intermodulation distortion products. Further evidence supporting this suggestion is discussed below. No sex differences in the shape/slopes of the curves describing the relationship between L1-L2 and DPOAE levels were observed, suggesting that the mechanisms involved in DPOAE generation may be the same for both sexes.

C. DPOAE audiograms

1. DPOAEs can be evoked in both papillae

The correspondence between frequency ranges to which nerve fibers are tuned and the frequency ranges for which relative maximum DPOAE amplitudes are recorded indicates that DPOAEs are produced in both papillae present in the frog inner ear (Figs. 6 and 7). Our findings are in agreement with previous results on R. p. pipiens (Van Dijk et al., 2002; Meenderink and Van Dijk, 2004). For the primary level tested in our DPOAE audiogram experiment (60 dB SPL), the emission levels recorded from the AP were higher than those recorded from the BP. Van Dijk et al. (2002) reported almost equal DPOAE levels from both papillae of R. p. pipiens for primary levels of 90 dB SPL. The difference in the results of the two studies probably reflects differences in the primary-tone levels used, an explanation supported by our study’s DPOAE I/O data (Fig. 2). More specifically, Fig. 2 indicates that, for low primary levels, DPOAE amplitudes recorded from the AP are higher than those recorded from the BP while, for high primary levels, the DPOAE amplitudes recorded from the AP and the BP are comparable.

2. Different generation mechanisms for the f1-f2 and 2f2-f1 distortion products

In mammals, the level of the DPOAE at 2 f1-f2 is typically plotted as a function of f2, reflecting very specific theoretical assumptions/hypotheses regarding the generation mechanism and place of origin of the 2 f1-f2 DPOAE. In studies on frog DPOAEs (Baker et al., 1989; Van Dijk and Manley, 2001; Van Dijk et al., 2002), DPOAE levels have been plotted as a function of f1, a choice based on convention (following Köppel et al., 1993). Due to the absence of a theoretical framework for DPOAE generation in the frog ear, we plotted DPOAE levels in three different ways.

The dashed vertical lines in Figs. 6 and 7 mark the frequency separation between the AP and BP for the species tested, as determined by neural tuning curves, and are therefore within a frequency range of low sensitivity. The assumption of lower sensitivity at the edges of a papilla’s frequency response versus the center is supported by the results of several studies (e.g., Ehret and Capranica, 1980; Narins and Hillery, 1983; Zelick and Narins, 1985). Since lower DPOAE levels and higher DPOAE thresholds have in general been measured at frequency ranges associated with the least sensitive hearing regions of the species examined (e.g., Probst et al., 1991), the degree of alignment between the DP audiogram dips and the vertical lines in Figs. 6 and 7 provides useful information regarding the site of DPOAE generation. For example, if a plot in Figs. 6 and 7 has its dip and vertical line misaligned, the expectation that emission levels should be minimal at the frequency range of minimal response is not satisfied. From this observation it can be deduced that the generation site of the respective distortion product cannot be related to the generation site of the frequency component against which the distortion product levels have been plotted. Figure 6 indicates that, for R. p. pipiens, alignment of the 2 f1-f2 DPOAE audiogram dip and the break in the frequency coverage between the two papillae (1250 Hz; Ronken, 1991) is best when 2 f1-f2 amplitude is plotted as a function of DP frequency [Fig. 6(c)]. This suggests that the generation of the 2 f1-f2 distortion product is related to the generation region corresponding to the DP frequency and not to that corresponding to the frequencies of the primaries. For the 2 f2-f1 DPOAE, on the other hand, alignment occurs when 2 f2-f1 amplitude is plotted as a function of f2 [Fig. 6(b)]. Near alignment is also observed when this distortion product is plotted as a function of f1 [Fig. 6(a)]. This suggests that, in contrast to 2 f1-f2, the generation of the 2 f2-f1 DPOAE is related to the frequencies of the primaries and not to the distortion product frequency. Similar results were obtained for R. catesbeiana (Fig. 7), supporting a similar interpretation.

The implication is that the generation of the 2 f1-f2 DPOAE in the frog may primarily occur at or near the DPOAE frequency place, while the generation of the 2 f2-f1 DPOAE may primarily occur at a frequency place between the two primaries. These results differ from those observed in mammals. As indicated by suppression studies using a variety of methods and confirmed by studies assessing recovery functions following short exposures to pure-tone or noise stimuli, the generation of the 2 f1-f2 DPOAE in mammals occurs primarily at a frequency place between the primaries. The distortion product itself then acts as a weak stimulus and may induce stimulus-frequency emissions at the distortion product frequency (Shera and Guinan, 1999). In contrast, the generation of the 2 f2-f1 DPOAE occurs at or near the
DPOAE frequency place on the basilar membrane (Probst et al., 1991; Knight and Kemp, 2000). The opposite behavior of the $2f_2-f_1$ and $2f_1-f_2$ DPOAEs observed between mammalian and frog DPOAE data may be related to various anatomical differences between the mammalian and frog ears and needs to be researched further.

Figures 6(c) and 7(c) also reveal that, for frequencies in the AP, the $2f_2-f_1$ and $2f_1-f_2$ curves are very similar in shape and overlaying when DPOAE levels are plotted against emission frequency. This suggests that, regardless of the place of generation and prior to their reemission from the AP, both DPOAEs may be subject to the same mechanical filtering by a structure with broad filter characteristics, possibly the tectorial membrane. Several studies have used DPOAE data to derive filter characteristics (e.g., Brown et al., 1992; Allen and Fahey, 1993; Kössl and Vater, 1996), while a recent study (Lukashkin and Russell, 2003) specifically argues for the existence of a possible tectorial membrane filter in mammals, based on the phase characteristics of different order DPOAEs.

3. Sexual dimorphism manifested in DPOAEs

Figures 9 and 10 indicate that, for the selected primary-tone levels, and over most of the frequency range tested, female subjects produced higher emission levels than male subjects in both species. Overall, R. catesbeiana produced higher emission levels than R. p. pipiens. In addition, there is a difference in the DPOAE audiogram peaks between males and females, suggesting that there are differences in the frequency tuning between sexes for both species.

Several studies discussed in Probst et al. (1991) have shown significantly higher levels of spontaneous OAEs for females in adult, children, and infant humans compared to males. The cause of this sex difference is not clear. In the female organ of Corti, OHCs are arranged much more irregularly than in males, possibly due to the smaller physical dimensions of the female cochlea. It has been argued that such irregularities may be linked to OAE generation (Lonsbury-Martin et al., 1988). McFadden and Pasanen (1998) linked EOA level differences between males and females to hormonal differences. They reported lower emission levels for homosexual than heterosexual females, and argued that the nature of this difference is consistent with the idea that homosexual females are exposed prenatally to higher levels of androgens than are heterosexual females.

Another possible explanation may lay in sex differences in middle ear transfer functions. In humans, for example, all OAEs can be detected reliably within a range (1–8 kHz) determined to a large extent by the frequency range (1–6 kHz) of effective reverse transmission from the middle ear (Probst et al., 1991). Puria (2003) argues that the forward and reverse transfer characteristics of the mammalian middle ear alter significantly the levels of DPOAEs recorded in the ear canal, in a frequency specific manner (higher frequencies are increasingly attenuated). Johansson and Arlinger (2002) recorded significantly higher DPOAEs from human female subjects and linked high middle-ear compliance present in males to low emission levels. Additionally, clinical OAE studies in infants routinely record higher evoked OAEs emissions from female newborns (e.g., Newmark et al., 1997), with this difference increasing with the frequency of the primaries (e.g., Cassidy and Ditty, 2001). The frequency-dependent change in EOAE levels may therefore be linked to the frequency-dependent transfer characteristics of the middle ear demonstrated by Puria (2003), which, as Johansson and Arlinger (2002) have argued in terms of middle ear compliance, may be sexually dimorphic.

In a recent study of bullfrogs, Mason et al. (2003) showed that, according to the current models of middle ear function, there is considerably higher middle ear impedance transform ratio in male bullfrogs compared to females. Although, according to the authors, it is not certain whether this reflects the assumptions of the model or actual physiological differences, such a transform ratio difference between male and female bullfrog middle ears corresponds well with the observed sex differences in the DPOAEs. In addition to middle ear transfer differences, sex differences in the frog have been reported in relation to peripheral auditory response. Narins and Capranica (1976) demonstrated such differences for the neotropical treefrog Eleutherodactylus coqui, associating sexual selectivity in the response properties of the BP to sexual selectivity in behavior. Since higher emission levels are thought to correspond to higher auditory sensitivity, frequency-dependent differences between sexes in OAE levels may indeed reflect sex differences in auditory sensitivity, possibly associated with male-female differences in what constitutes a biologically relevant sound.

V. SUMMARY AND CONCLUSIONS

DPOAE measurements were made for the $2f_1-f_2$ and $2f_2-f_1$ distortion products from male and female R. p. pipiens and R. catesbeiana. DPOAE I/O curves were obtained at frequencies corresponding to the best response in the AP and BP of both species. The $2f_1-f_2$ I/O curves obtained from the AP of both species are very similar to mammalian DPOAE I/O curves, exhibiting a non-monotonic response, with a distinct notch for primary levels ~70 dB SPL. This similarity suggests that, like in the mammalian ear, there may be an amplification process present in the frog AP manifested as a compressive nonlinearity with saturation. In mammals this process is linked to prestin-mediated OHC activity. Since the frog ear lacks OHCs and its hair cells contain no prestin in their basolateral membranes, an alternative amplifier may be based on hair cell bundle spontaneous movement, shown to occur in the frog sacculus. The DPOAE I/O curves obtained from the BP of both species exhibit a rather monotonic response, suggesting that no amplification process may be present in this papilla. The difference in the response between amphibian and basilar papilla may be linked to respective differences in degree and type of innervation. It is also consistent with the fact that only the AP is tonotopically organized and could take better advantage of an amplification mechanism that increases tuning sharpness, as well as with the observation that only this papilla exhibits SOAEs.

The dependence of DPOAE level on the relative levels of primaries ($L_1-L_2$) was investigated in $R. p. pipiens$, within the range $50 \leq L_1, L_2 \leq 60$ dB SPL. Consistent with intermodulation distortion expectations alone and differently
from mammalian results, highest emissions were generally obtained for \( L_1 \approx L_2 \). This difference between mammalian and frog data is consistent with a difference in neural tuning-curve shape of mammalian and frog nerve fibers with comparable characteristic frequencies. In the mammalian case, neural tuning curves are thought to reflect BM displacement envelopes, which are in turn considered responsible for the dependence of DPOAE level on the relative levels of the primaries. Since the frog ear lacks a BM, the observed DPOAE level dependence on \( L_1 - L_2 \) in the frog suggests that, if some mechanical structure (i.e., the tectorial membrane) functions analogously to the mammalian BM, it must be more broadly tuned. Our interpretation is supported by the rest of our results, outlined below, and does not exclude the possibility of the optimal primary-level difference to be \( L_1 - L_2 \neq 0 \) for primary levels outside the level-range examined.

DPOAE audiograms were obtained from males and females of both species for primary frequencies spanning the animals’ hearing range and primary levels determined by the present study. The results indicate the following:

(a) In agreement with previous DPOAE frog results (Van Dijk et al., 2002; Meenderink and Van Dijk, 2004), DPOAEs are produced in both frog papillae and, except for primary frequencies at the high-frequency end of the BP, *R. catesbeiana* produce stronger emissions than *R. p. pipiens*.

(b) In contrast to mammalian results, the generation of the 2\( f_1 - f_2 \) DPOAE in the frog appears to occur primarily at or near the DPOAE frequency place, while the generation of the 2\( f_2 - f_1 \) DPOAE occurs primarily at a frequency place between the primaries. Further study is needed to confirm the observed difference in DPOAE generation sites between mammals and frogs and determine the precise reason behind it. Regardless of DPOAE generation site, both DPOAEs within the AP may be subject to the same filtering mechanism, possibly the tectorial membrane.

(c) For the primary-tone levels used, females of both *R. p. pipiens* and *R. catesbeiana* produce higher emissions than males in both papillae, with the difference being larger in the BP. The observed similarities in male and female frog DPOAE data in terms of I/O curve shapes, dependence on \( L_1 - L_2 \), etc. suggest that sex differences in overall DPOAE levels are not related to DPOAE generation mechanism differences. DPOAE-based sexual dimorphism, also reported in humans and other mammals, may be linked to inner ear anatomical differences, middle ear transfer impedance differences, hormonal differences, nerve-fiber tuning differences, and/or behavioral and environmental factors.

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\[1\] In frogs, OAEs have been recorded from the ears of *Rana pipiens pipiens* (DPOAEs: Van Dijk et al., 2002; Meenderink and Van Dijk, 2004; spontaneous OAEs (SOAEs): Van Dijk et al., 1996). *Rana catesbeiana* (SOAEs: Gennona et al., 1989), *Rana esculenta* (SOAEs: Palmer and Wilson, 1982; Van Dijk et al., 1989; Van Dijk and Wit, 1987; stimulus-frequency OAEs (SFOAEs): Palmer and Wilson, 1982), *Rana temporaria* (SOAEs: Baker et al., 1989), *Xenopus laevis* (DPOAEs: Van Dijk et al., 2002), *Hyla cinerea* (DPOAEs: Van Dijk and Manley, 2001; SOAEs: Van Dijk et al., 1996), *Hyla chrysoscelis*, *Hyla versicolor*, and *Leptodactylus albilabris* (SOAEs: Van Dijk et al., 1996). No measurable SOAE levels were obtained from *Xenopus laevis* (Van Dijk et al., 1996) and no measurable DPOAE levels were obtained from *Scaphiopus couchi* (Van Dijk et al., 2002). Neither SOAEs nor DPOAEs could be measured from *Bombina orientalis* (Van Dijk et al., 1996 and Van Dijk et al., 2002 respectively).

\[2\] E.g., \( f_1 f_2 = 1.2 \) in Kalluri and Shera (2001); \( f_1 f_2 = 1.22 \) in Emmerich et al. (2000); \( f_1 f_2 = 1.25 \) in Kemp and Brown (1983) (in Probst et al., 1991).

\[3\] The only available parametric DPOAE study on the frog to date (Meenderink and Van Dijk, 2004) examines in detail the I/O characteristics of the two papillae in *R. p. pipiens*, offering results that, in general, agree with the results of the present study.

\[4\] Although exploration of the \( f_1 f_2 \) space in mammals has revealed several discontinuities, we assume that the value selected by the present study is close enough to the value used in previous frog DPOAE studies to allow for a meaningful comparison.

\[5\] The body temperature of the animals was not monitored systematically.

\[6\] Below 200 Hz the noise floor of the probe/analysis system was too high (>25 dB SPL) and the speaker response too weak (<65 dB SPL), while the hearing range of neither frog species examined exceeds 3000 Hz.

\[7\] Windowing: Blackman and Harris; averaging: 200 time frames with 90% overlap; input range: adjusted for each primary tone level combination to ensure consistent use of the analyzer’s full dynamic range; harmonic/intermodulation distortion of the speaker–microphone–analyzer system >83 dB below the primaries at all frequencies tested. System distortion was determined by attaching the probe tube system to a hard surface and measuring the level recorded at frequencies corresponding to DPOAE frequencies for selected primary-tone frequency and amplitude values. The dBV reading of the analyzer was converted to dB SPL using a measuring amplifier (B&K 2609, Bruel & Kjær, Nærum, Denmark).

\[8\] The analysis bandwidth was 0.98 Hz for 240 Hz <\( f_1 < 800 \) Hz, 1.95 Hz for 850 Hz <\( f_1 < 1600 \) Hz, and 3.9 Hz for 1700 Hz <\( f_1 < 3000 \) Hz.

\[9\] Shofner and Feng (1984) indicate 1000 Hz as the frequency value marking the separation between AP and BP response in *R. catesbeiana*.

\[10\] One alternative hypothesis involves transition among different vibration modes of the inner ear structures as the levels of the primaries change, claimed to be responsible for the observed DPOAE I/O curve notch. In such an explanation, it is expected that, for a given set of primary tone frequencies, the DPOAE I/O curve notches should be observed at the same stimulus levels, regardless of distortion product. Our data indicate that this may be the case for the *R. catesbeiana* I/O curves but it certainly is not for the *R. p. pipiens* I/O curves [Figs. 2(a) and 2(c)], suggesting that the “vibration mode transition” hypothesis may be excluded as an explanation for the DPOAE I/O curve shapes observed. Another explanation for the presence of notches in the DPOAE I/O curves is based on a hypothesized frequency shift of DPOAE fine-structure in human subjects with changing stimulus levels (He and Schmidt, 1993; Whitehead, 1998). Figure 3 demonstrates that this is not the case for the frog species tested. In mammals, the presence of AP audiogram fine structure has been observed in terms of interference between two emission components (e.g., Talmage et al., 1998, 1999). Further study is needed to determine the origin of the DPOAE fine structure observed in the frog.

\[11\] In the case of the frog ear, however, the DPOAE phase data obtained and analyzed by Meenderink and Van Dijk (2004) do not appear to support such a model.

\[12\] The presence of an inner ear amplifier in the echidna, a Monotreme with features common to early mammals, birds, and reptiles and which has no organ of Corti, has also been inferred based on DPOAE data (Mills and Shepherd, 2001).

\[13\] The range of primary stimulus levels tested (50–60 dB SPL) is below the DPOAE I/O curve saturation point for *R. p. pipiens* (Fig. 2), supporting the suggestion that the observed effect of \( L_1 - L_2 \) on DPOAE levels is indeed indicative of a difference between mammalian and frog responses as opposed to simply a level saturation response in the frog.

\[14\] In an alternative interpretation of the data by Hillery and Narins (1987, Pitchford and Ashmore (1987) argue that the large phase accumulations in the phase-locked responses of auditory-nerve fibers in frogs may not reflect phase accumulations of a mechanical traveling wave (e.g., Hillery
19 Sex differences in DPOAE phase delay measures have also been attributed to such factors. Although the dependence of DPOAE levels on animal weight and/or the size of the eardrum was not systematically examined, our data suggest that any DPOAE level difference between species and sexes is most likely not related to such factors.

18 Sex differences in DPOAE phase delay measures have also been attributed to anatomical differences in cochlear length (Bowman et al., 2000).


