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Distortion Product Otoacoustic Emission and Auditory Brainstem Responses in the Echidna (*Tachyglossus aculeatus*)

David M. Mills[1] and Robert K. Shepherd[2]

[1]*V. M. Bloedel Hearing Research Center, Departments of Otolaryngology, Head and Neck Surgery, University of Washington, Seattle, WA 98195, USA*
[2]*Department of Otolaryngology, University of Melbourne, Melbourne, Australia*

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ABSTRACT

The auditory function of four wild-caught echidnas was measured using distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs). Emission audiograms were constructed by finding the stimulus levels required to produce a criterion emission amplitude at a given stimulus frequency. For an emission amplitude of −10 dB SPL, the median "best threshold" was 28 dB SPL, and this minimum threshold occurred between 4 and 8 kHz for all animals. The *relative* effective range of auditory function was defined by the frequencies at which the audiogram was 30 dB above its best threshold. For the emission audiograms, the median lower-frequency limit was 2.3 kHz, the upper limit was 18.4 kHz, and the effective range was 2.7 octaves. The audiogram as measured by ABR was also found to be strongly "U" shaped with similar low- and high-frequency limits, i.e., from 1.6 to 13.9 kHz, with an effective range of 3.1 octaves. These results suggest that the echidna has a behavioral hearing sensitivity comparable to that of typical therian mammals (e.g., rabbits and gerbils) but with a significantly narrower frequency range. DPOAE responses were also measured in selected animals as a function of the variation of all four stimulus parameters (frequencies and intensities of both stimulus tones). Overall, the measured emission responses establish that the echidna does have a cochlear amplifier, and that it *could* be the same type as in therian mammals. The amplification mechanism in the echidna, currently unidentified, clearly operates to frequencies above 20 kHz, higher than the hearing function observed in any birds or reptiles but lower than for typical therian mammals. This raises the possibility that at least some aspects of the mammalian cochlear amplifier developed early in evolution, before the divergence of the monotremes (echidna and platypus) from the mainstream therian mammals (marsupials and placentals). In this respect, the presence or absence of outer hair cell electromotility in monotremes would have important consequences for understanding the function and evolution of the vertebrate inner ear.

Keywords: peripheral auditory system, evolution, cochlear amplifier, mammal, monotreme, DPOAE, ABR

INTRODUCTION

The echidna is one of only three surviving species that compose an entire order, the Monotremata (reviews: Griffins 1968, 1978; Augee and Gooden 1993; Grant 1995). Monotremes occupy a unique position among vertebrates, having many features common to other mammals (therian mammals) but also features common to early mammals and birds and reptiles (review: Carroll 1988). Their peripheral auditory system shows a similar mix of features. The monotreme middle ear is typically mammalian, consisting of three bones with the same functions as in other extant mammals (Griffiths 1968; Aitkin and Johnstone 1972; Gates et al.

Correspondence to: David M. Mills, Ph.D. • University of Washington • Box 357923 • Seattle, WA 98195. Telephone: (206) 616-7540; fax: (206) 221-5685; email: dmmills@u.washington.edu

1974). However, the monotreme inner ear is not typically mammalian. All extant therian mammals, i.e., all modern marsupials and placentals, have a cochlear duct which wraps into a spiral of 2–4 turns (e.g., Luo and Ketten 1991; reviews: Pickles 1988; Echteler et al. 1994; Aitkin 1995, 1998). In contrast, the monotreme cochlear duct curves only slightly and, moreover, contains a lagenar macula (Chen and Anderson 1985; Joørgensen and Locket 1995; Ladhams and Pickles 1996). In this respect, its structure is similar to that in early mammals and in birds and reptiles (e.g., Allin 1986; Rosowski 1992; Luo et al. 1995; Hu et al. 1997; reviews: Webster et al. 1992; Fox and Meng 1997; Dooling et al. 2000; Gleich and Manley 2000). Moreover, while the monotreme cochlea does have an organ of Corti, there are about twice the number of hair cells and support cells across the organ compared to therian mammals (Ladhams and Pickles 1996). These observations, together with other fossil and molecular evidence, suggest that monotremes diverged very early from the line leading to modern therian mammals, well before the divergence between the placentals and marsupials (e.g., Messer et al. 1988; Carroll 1988; Luo and Ketten 1991; Luo et al. 2001).

Therefore, it is of interest to determine whether the monotreme hearing system is more like that of other mammals or more like that of birds and reptiles and to investigate the possibly unique mechanisms by which its inner ear functions. Note in this respect that the *typical* therian mammalian hearing system (excluding humans and chimpanzees) functions well up to 30–50 kHz and that some bats and marine mammals have excellent hearing above 100 kHz (Brown 1973; Reimer 1995; Aitkin et al. 1996; Cone-Wesson et al. 1997; reviews: Fay 1988, 1994; Echteler et al. 1994; Kössl and Vater 1995; Popper and Fay 1995). No birds have developed sensitive hearing above 12 kHz, even animals with specialized auditory systems, e.g., barn owls (Konishi 1973), where this might be an advantage to the animal. The cat, for example, which uses its hearing to locate some of the same prey species as the barn owl, has developed excellent, very sensitive hearing that extends well above 40 kHz (Liberman 1982). The attainable upper frequency range in the auditory system is the major obvious functional difference between therian mammals and all other vertebrates (reviews: Fay 1988; Manley 1990; Dooling et al. 2000).

This difference in the upper frequency limit has been attributed to a limitation of the simple, columella middle ear of birds and reptiles compared to the three-bone system in mammals (e.g., Manley 1972a, b, 1990; Gummer et al. 1989a, b; review: Saunders et al. 2000). However, it is possible that at least some of the differences in high-frequency response arise from the existence of different inner ear mechanisms in birds and reptiles compared to mammals (e.g., Manley 1972a, 2000). Since monotremes do have an essentially modern mammalian middle ear (Griffiths 1968; Fleischer 1978) but an inner ear in some respects similar to that of birds and reptiles, it seems important to determine where the monotreme frequency response falls in the spectrum between birds and therian mammals.

In this regard, it is not known at all if monotremes have a "cochlear amplifier" and, if so, what mechanisms are employed for its function. In therian mammals, the cochlear amplifier refers to a set of physiologically vulnerable processes that act to physically amplify the passive cochlear traveling wave at low stimulus levels (Davis 1983; Ruggero and Rich 1991; Cody 1992; Russell and Nilson 1997; Rhode and Recio 2000; reviews; Patuzzi and Robertson 1988; Dallos 1992). There also appears to be effective amplification of the acoustic stimulus in birds and reptiles which, however, does not appear to involve the same kind of amplification of a macroscopic traveling wave (reviews: Popper and Fay 1999; Gleich and Manley 2000; Manley 2000). All of these vertebrates produce otoacoustic emissions which have generally similar characteristics (e.g., Norton and Rubel 1990; Taschenberger et al. 1995; Taschenberger and Manley 1998). The detection of otoacoustic emissions at relatively low stimulus levels has been considered evidence of the presence of nonlinear amplification processes in the inner ear since their discovery (Kemp 1978; Brown et al. 1989; Johnstone et al. 1990; Manley et al. 1993; Mills 1997).

Given the potential significance of the monotreme auditory system, it is noteworthy that there have been no functional observations published in the 30 years since the first measurements were reported (Griffiths 1968; Aitkin and Johnstone 1972; Gates et al. 1974). No types of otoacoustic emissions nor of auditory brainstem responses have ever been measured in a monotreme. In fact, there have been no noninvasive hearing tests of any monotreme. In this regard, note that only relatively recently was it discovered that the sensitive function of the mammalian cochlea was extremely vulnerable to typical invasive experimental manipulations (Sellick et al. 1982). This includes the effect of cooling the exposed cochlea in typical laboratory measurements (Brown et al. 1983). In retrospect, many earlier measurements had to reinterpreted when it was realized that these experiments had damaged the cochlear amplifier to the extent that they actually had been measuring only passive cochlear function (e.g., von Békésy 1960).

By design, the present study of hearing function in the echidna was limited to noninvasive techniques that could be employed in lightly anesthetized animals. This had the advantages that (1) it minimized the potential for damaging cochlear function by the measurement process, (2) allowed the hearing function to

be as normal as possible, and, (3) not least, allowed the release of the wild-caught animals after measurement. All animals were measured using both auditory brainstem responses (ABRs) with tone pips and distortion product otoacoustic emissions (DPOAEs) with continuous tones. In addition, tympanographic measurements were made in selected animals.

METHODS

Four adult echidnas (*Tachyglossus aculeatus*) were captured in the Strathbogie range north of Melbourne. Weights ranged from 3 to 4.5 kg. The exact location of capture was noted and, after completion of measurements and complete recovery from anesthesia, each animal was returned to the place from which it was collected. Animals to be studied were housed as a group in a large animal room at Monash University and given free access to food and water. Prior to measurement of auditory function, animals were anesthetized with an intraperitoneal (IP) injection of a combination of ketamine (12 mg/kg) and xylazine (1 mg/kg). Following induction, supplemental doses were given IP or intramuscularly at intervals of one-half to one hour, as required to maintain a condition of adequate relaxation. All procedures were noninvasive. After initial anesthesia, both outer ear canals were inspected otoscopically. Usually ear canals were found to be clean and dry, except occasionally ticks were found and were removed. However, it was not possible to visualize the tympanic membrane, even with an endoscope. For this reason, the tympanic membrane and middle ear air pressure were investigated using a tympanometer (GSI 28 Auto Tymp) in selected animals. The tympanic compliance was found to have a maximum near atmospheric pressure, suggesting that normal (i.e., near atmospheric) middle ear pressure was maintained under the anesthesia protocol employed.

All emission and ABR measurements were conducted in a sound isolation room at the Royal Victorian Eye and Ear Hospital in Melbourne. The animal was placed on its abdomen on a heating pad and the temperature of the abdominal skin maintained at 33°C. The room temperature was 24–26°C. Internal temperature probes were not used to avoid any possibility of damage to the cloaca. The main goal of the temperature maintenance for the echidna was to insure that the animal was not overheated. The echidna internal temperature is typically 32°C or less, and elevations to 34°C can be fatal (Grigg et al. 1992). With the procedures used, the echidnas appeared to temperature regulate well. They did not appear to overheat nor did they go into a dormant state, as evidenced by the fact that they recovered quickly when removed from the table on completion of measurements. Perhaps the best indication of the stability of the preparation was that emission amplitudes were typically stable within 1 dB throughout the measurements. Their stability was equal or superior to emission measurements made over similar time periods in laboratory animals under more rigidly controlled conditions, e.g., in gerbils where the internal temperature was closely controlled and the bulla opened so that middle ear pressures were maintained at exactly atmospheric pressure (e.g., Mills and Rubel 1994, 1996).

For ABR and emission measurements, the ear canal was gently opened and the acoustic coupler (Mills and Rubel 1996) placed carefully into the outer ear canal. If there was a reaction in the muscles which act to close the pinna and ear canal, supplemental anesthesia was given and coupler insertion delayed until there was an absence of obvious reflex to touching the ear canal. Usually only one ear was measured in a given session. Except during induction, the animal was deliberately kept only lightly anesthetized.

Equipment and procedures for emission and evoked potential measurements were similar to those previously reported (Mills and Rubel 1996; Mills 2000). Briefly, a closed acoustic system—an acoustic coupler sealed to the ear canal—was employed for all measurements. Tones or clicks were produced by loudspeakers in custom enclosures and the sound led to the ear canal through tubes into the coupler. The sound pressure level at the entrance to the ear canal was measured by a low-noise microphone (ER-10B, Etymotics, Elk Grove Village, IL) also joined to the coupler. In some animals, the output of the low-noise microphone was calibrated by reference to the output of a probe tube microphone located in the same coupler (Mills and Rubel 1996). For the echidna, the correction required up to 24 kHz was found to be small and very consistent, and there was no correction required up to 16 kHz. While the measurement system was designed for frequencies to 50 kHz, the maximum frequency for echidna hearing was found to be about 20 kHz. Therefore, for most of the measurements in this study, the probe tube microphone was removed to reduce the overall coupler size and facilitate the fit into the ear canal.

A basic set of distortion product otoacoustic emission (DPOAE) measurements was obtained first. Only two-tone measurements (frequencies f_1 and f_2, $f_1 < f_2$) were used in these studies. In all ears tested, an emission "audiogram" was determined as follows. Input–output ("growth") functions were measured by fixing the two stimulus frequencies and the ratio of their stimulus levels and stepping the two stimulus levels upward in increments of either 3 or 5 dB. The parameter set included the frequency ratio $f_2/f_1 = 1.21$ and the level ratio $L_1/L_2 = 10$ dB. In all cases, the f_2 frequencies were stepped up from 1 kHz at an interval of 0.5 octave.

In addition, approximate thresholds were determined online, and additional frequencies were chosen when the thresholds were found to be changing rapidly. Either immediately before or after the animal experiment, instrumental distortion levels were estimated. This procedure involved running the same series of frequencies and levels, but with a long tube (6 mm i.d. × 2 m) replacing the echidna ear canal (Mills and Rubel 1996). Typical results for measured growth functions are presented in Figure 1 for both animal measurements and instrumental distortion estimates.

Offline, each growth function was plotted and the emission audiogram constructed by plotting the stimulus level L_2 required to achieve a criterion level for the amplitude of the emission at $2f_1-f_2$ as a function of f_2 frequency. Threshold definitions for construction of emission audiograms are also illustrated in Figure 1.

In most animals, additional emission measurements were obtained following the establishment of the basic emission audiogram, in some cases following the ABR measurements discussed below. The additional emission measurements usually included "frequency ratio functions," i.e., measurements of the emission amplitude as the frequency ratio f_2/f_1 was varied from 1.03 to 1.9 in steps of about 0.02 at fixed stimulus levels. In addition, "level ratio functions" were also obtained in some animals by fixing the stimulus level L_1 and varying L_2 with fixed stimulus frequencies.

Audiograms were also obtained using auditory brainstem responses (ABRs) evoked by tone bursts. Parameters and techniques were similar to those reported elsewhere (Shepherd and Martin 1995; Aitkin et al. 1996). For the echida, stainless steel electrodes were placed in the midline, inserted through the skin and fat layer on the dorsal surface about 4 cm and 11 cm back from the "forehead," i.e., the point where the snout joined the head. Sound was delivered using the same coupler and calibration procedure established by the emission program. ABRs were recorded differentially against a ground electrode placed in the abdominal muscle. Responses were amplified by 100 dB, bandpass filtered (150 Hz to 3 kHz) and digitized using the second channel of the computer A/D input.

For all ears, ABR responses to clicks (100 μs rarefaction/condensation) were measured first, followed by responses to tone bursts. As with emission measurements, tone burst frequencies were chosen at 0.5-octave intervals or smaller. For all tone bursts, there was a $\cos^2$-shaped rise time of 1 ms, followed by 3-ms constant tone, then a 1-ms $\cos^2$ fall time. The repetition rate was 30 Hz. This is well within the range that was shown by Corwin et al. (1982) to have no effect on the ABR of most verterbrates, even those with low body temperatures. For all measurements, responses to 500 successive pips of alternating polarity were averaged. Two recordings were always made at the same stimulus parameters. For each tone pip or click waveform the stimulus intensity was reduced until the response became indistinguishable from the background. Thresholds were determined visually offline by superimposing successive recordings at the same levels (e.g., Aitkin et al. 1996). Typical results for a tone pip ABR measurement and its threshold determination are illustrated in Figure 2.

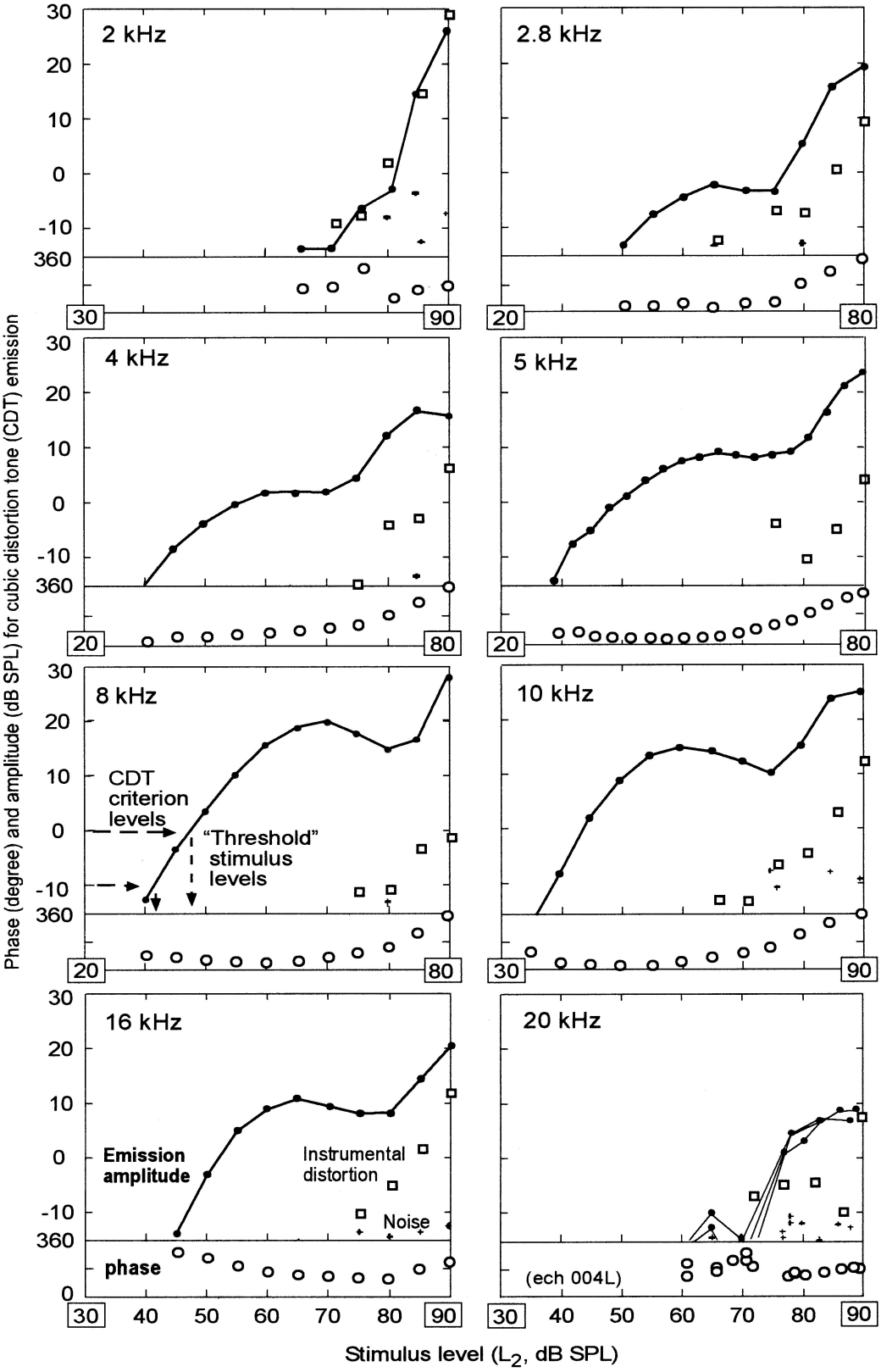
A permit for live capture and holding of echidnas was obtained from the Victorian Department of Natural Resources and Environment (to J. Nelson) and measurement procedures were approved by the Animal Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital, Melbourne, and the Animal Care Committee at the University of Washington, Seattle.

RESULTS

ABR and emission audiograms

Figure 1 presents a sample of emission "growth" functions measured for one ear (echidna 004L). These functions are plots of the cubic distortion tone (CDT) emission at $2f_1-f_2$ as a function of stimulus level for fixed stimulus frequencies. The stimulus levels L_1 × L_2 were varied together so that L_1 was 10 dB higher than L_2. The parameter noted in each panel is the stimulus frequency f_2. The frequency ratio for all growth functions in Figure 1 was $f_2/f_1 = 1.21$. "Emission audiograms" were constructed offline by choosing emission criterion levels as illustrated in Figure 1 (8-kHz panel), and plotting the associated L_2 "threshold" levels versus the f_2 frequency. Such audiograms are shown in Figure 3 for all six ears for which results were obtained in this study. For each animal, emission audiograms are shown for criterion levels of emission amplitude equal to 0, −5, and −10 dB SPL, as noted in the key in the upper-left panel. Similarly, a typical variation in ABR response is shown in Figure 2, with threshold determinations made as discussed in the Methods section. The ABR threshold audiograms found for all ears tested are also presented in Figure 3, indicated by the dashed lines and open circles.

While there are clearly some differences, both the ABR and emission audiograms generally agreed very well. Both indicate that the echidna audiogram was strongly U-shaped. The lower limit of auditory function was about 2 kHz in all animals. Their audiograms were always most sensitive between 4 and 8 kHz. Thresholds were quite similar among all animals tested given the fact that they were wild caught and of indeterminate ages. Among five ears, three had minimum ABR thresholds of 45 dB SPL, one was 40 dB SPL, and one was 50 dB SPL. The exception was echidna 003



(right column, middle row). The right ear of this animal (not shown) was not responsive to either emission or ABR measurements to 110 dB SPL, measured on two occasions. The sensitivity of the left ear varied somewhat as measured in three different sessions. Results from the best (most sensitive) session are

shown in Figure 3. These thresholds were elevated in both ABR and emissions, with the most sensitive ABR threshold equal to 65 dB SPL for this ear.

Tympanographic measurements in this animal did not indicate any obvious middle ear pathology. That is, there was a clear peak of tympanic compliance occurring near atmospheric pressure. On the other hand, the elevated threshold seen in Figure 3 did not have responses of typical cochlear hearings loss, i.e., predominantly high-frequency loss. Of course, nothing is known about how a "typical" echidna cochlear hearing loss would manifest, so the reasons for the elevated thresholds in one animal must remain unresolved. Even with these uncertainties, note that the *shapes* of the audiograms in the responsive ear of this animal were similar to all the other ears tested.

While the absolute threshold of hearing is not well established by either of the methods used, one can nonetheless define a *relative* effective width of an audiogram by the frequencies at which the threshold function rises above the lowest ("best") threshold by a criterion amount. Here, we chose the level that was 30 dB higher than the best threshold. For the five normal ears in Figure 3 (i.e., excluding echidna 003L), median values of the effective range of hearing are summarized in Table 1.

The ABR results agree well with the emission (DPOAE) estimates, shifted slightly downward in frequency. Overall, one can conclude from these results that the effective range of auditory response of the normal echidna extends from approximately 2 to 16 kHz, with a total extent of only 3 octaves.

Emission growth functions

Typical emission growth functions in the echidna are presented in Figure 1. As a function of the stimulus levels, the amplitude of the emission typically rose steeply from the noise floor at low levels; it "saturated" at midlevels so that it remained approximately constant across a range of stimulus levels; and only at very high levels did the amplitude increase sharply again. The saturation region was typically quite extensive and often

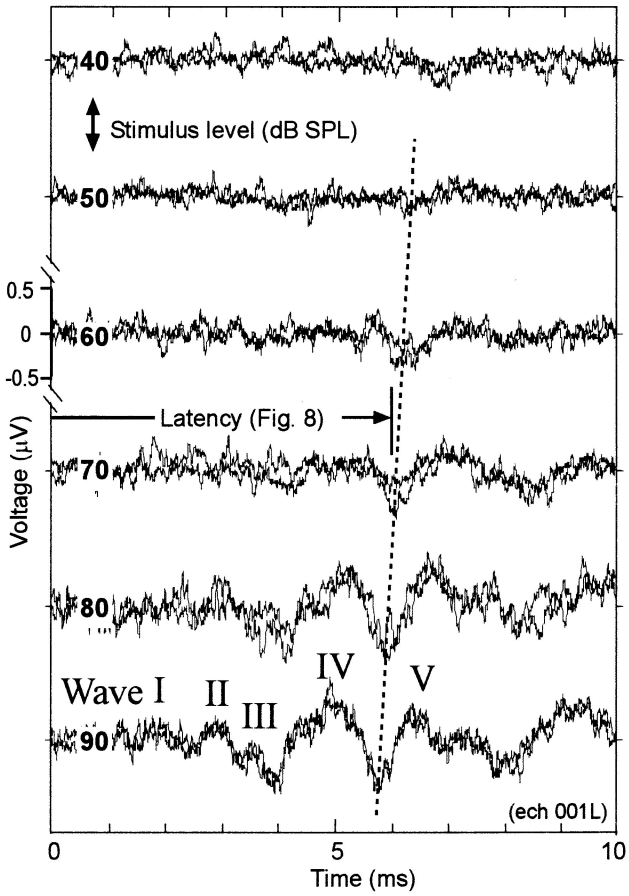


FIG. 2. Typical auditory brainstem responses (ABR). Stimulus was a ramped tone pip at 8 kHz, with other parameters as noted in the Methods section. The horizontal axis gives elapsed time with reference to the measured start of the ramp at the location of the microphone. The vertical axis is the averaged recorded voltage in μV, with vertex positive plotted upward. The stimulus level is noted in the left part of each curve, in dB SPL. In this example, threshold was determined to be 50 dB SPL. Note that the stimuli were first presented at 10-dB intervals from 40 to 90 dB SPL, then at 10-dB intervals from 45 to 75 dB SPL (not shown). Two runs were recorded at each level. The first five positive peaks have been identified using Roman numerals. For comparison with other latencies, the ABR latency was defined by the negative trough of wave IV, as indicated by the dashed line.

FIG. 1. Typical emission input–output, or "growth" functions, recorded in one animal. The horizontal axis is the stimulus level L_2 (dB SPL). Note that, as indicated by the boxed numbers, the horizontal axis for some panels extends from 20 to 80 dB SPL and for others from 30 to 90 dB SPL. For all of these growth functions, the lower-frequency stimulus was 10 dB higher than the upper, denoted L_1/L_2 = 10 dB. The vertical axis represents the emission at $2f_1-f_2$, denoted as the cubic distortion tone (CDT) emission. The stimulus frequency f_2 is indicated in the upper corner of each pair of panels. Solid lines illustrate the CDT amplitude in dB SPL, as noted in the key in the 16-kHz panel. Open circles represent the phase angles of this emission as measured at the low-noise microphone, relative to the stimulus phase. Crosses denote the noise floor, conservatively estimated by taking an rms average of the bins next to the CDT frequency. Open squares indicate the instrumental distortion amplitude for the same stimulus conditions, estimated by measuring the CDT emission amplitude with the animal replaced by a long tube joined to the coupler (see Methods section). Phase angles for instrumental distortion measurements are not shown but noise level estimates are. For reference, a key in the panel for the 8-kHz growth function indicates two of the levels chosen to represent emission "thresholds", shown in Fig. 3.

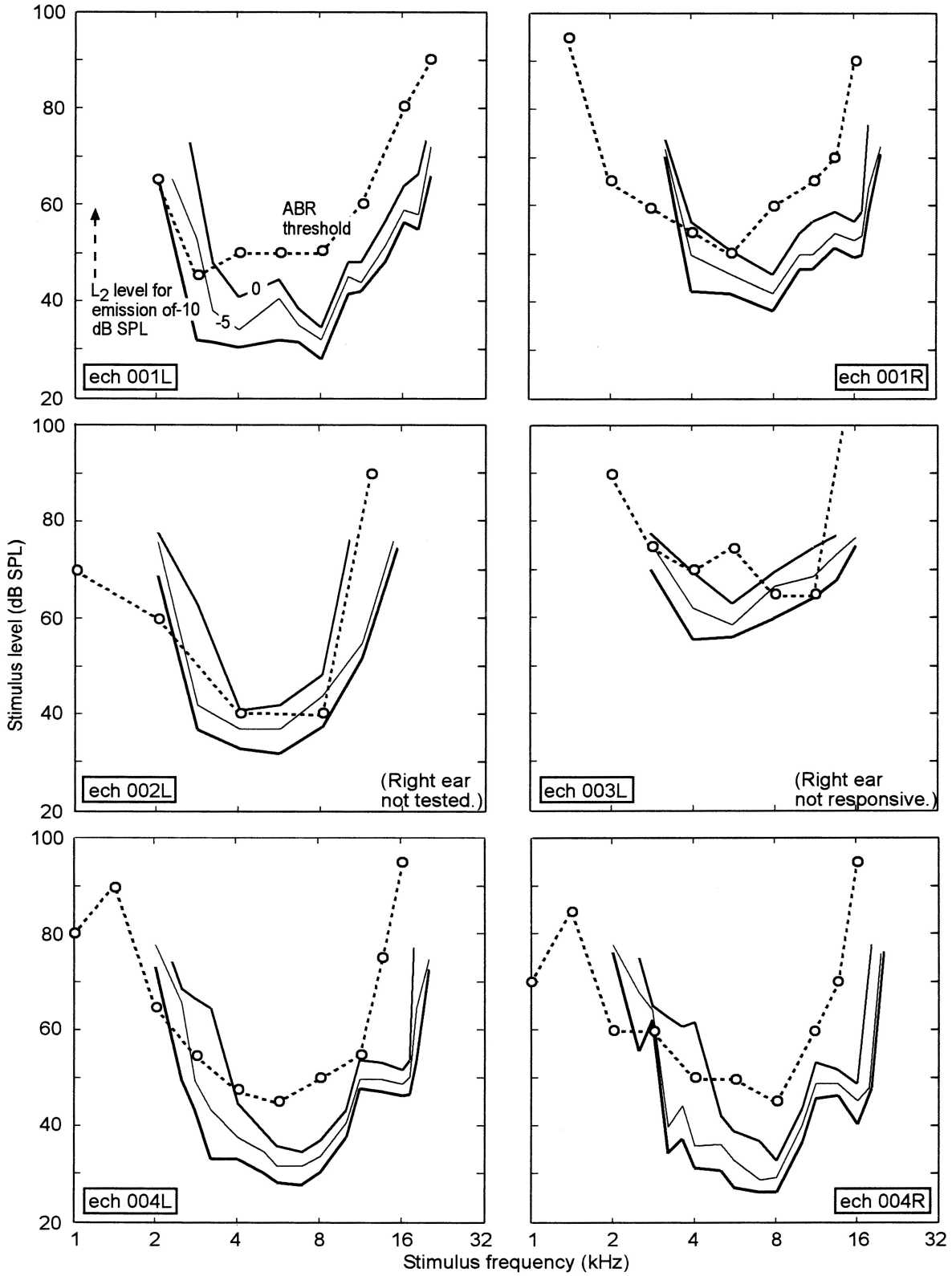


FIG. 3. Summary of the threshold curves obtained for all animals in the study. When threshold curves were determined for a given ear on more than one occasion, the most sensitive set was chosen for presentation. The set of ABR and emission thresholds shown were always those recorded in the same session, and the stimulus parameters were the same for all panels. The horizontal axis in each panel gives the appropriate stimulus frequency, i.e., that of the tone pip for the ABR and that of the higher-frequency stimulus (f_2) for the emission measurements. The vertical axis in each panel represents the threshold stimulus level as defined in Figs. 1 and 2, i.e., the tone pip stimulus level for the ABR and the stimulus level L_2 for the emission measurements. As indicated in the key in the upper-left panel, the heaviest solid line indicates the emission threshold L_2 for a criterion amplitude for the CDT equal to −10 dB SPL. Lighter lines show thresholds for levels of −5 and 0 dB SPL. ABR thresholds are represented by the open circles and dashed lines.

TABLE 1

Echidna auditory function[a]

Measurement technique	*Best threshold (dB SPL)*	*Low-frequency limit (kHz)*	*High-frequency limit (kHz)*	*Effective range (octaves)*
DPOAE	28.0 (26.7–33.5)	2.3 (2.1–2.9)	18.4 (17.0–18.6)	2.7 (2.6–3.0)
ABR	45.0 (43.7–46.3)	1.6 (1.4–1.8)	13.9 (12.8–14.9)	3.1 (3.0–3.3)

[a]Each entry represents the median value, with the 25th and 75th percentiles (interquartile range) in parentheses, for the five normal ears shown in Fig. 3 (i.e., excluding echidna 003L). The low- and high-frequency "limits" listed represent the *relative* effective range of hearing, defined by those points in the audiogram that are 30 dB up from the best threshold. For distortion product otoacoustic emission (DPOAE) measurements, the "threshold" audiograms were those defined by the levels L_2 at which the emission at $2f_1-f_2$ equaled −10 dB SPL (lowest solid lines, Fig. 3). Auditory brainstem response (ABR) audiograms were determined as illustrated in Figs. 2 and 3.

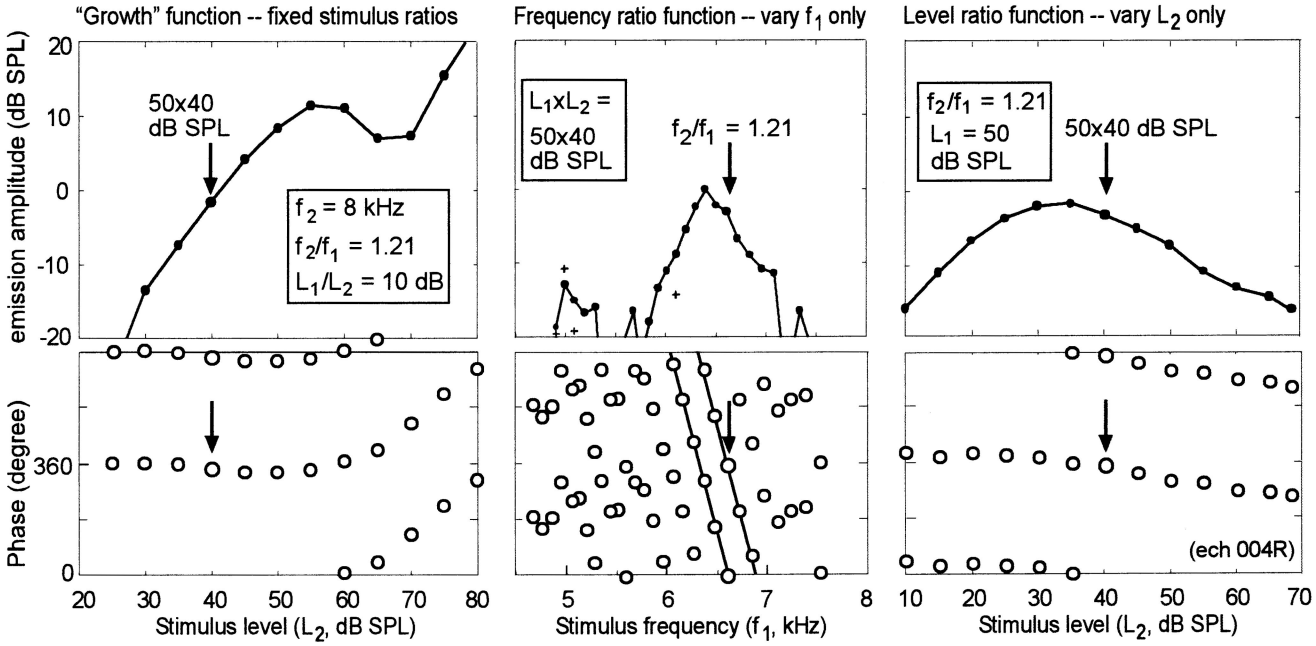


FIG. 4. Comparison of emission responses with three different methods of varying parameters, measured in one session. For each pair of panels, the upper panel shows the emission amplitude response and the bottom panel shows the phase response for a given method. The leftmost pair of panels shows a growth function, i.e., the variation with stimulus levels for fixed stimulus frequencies and fixed stimulus level ratio, in this case L_1/L_2 = 10 dB. Other growth function responses are shown in Fig. 1. The middle column shows the variation with frequency ratio f_2/f_1, in this case the "frequency ratio function" defined by fixed stimulus amplitudes ($L_1 \times L_2$) and fixed f_2 frequency. The right column shows the variation with stimulus level ratio while holding the stimulus frequencies and one stimulus level (L_1) constant. The vertical arrow in each panel indicates the three measurements that had the same nominal parameters but which were taken at different times in the same session. For clarity, phase angles (lower panels) are repeated at 360° intervals. The lines in the lower center panel indicate the slope of the phase shift which is used to calculate latencies, presented in Fig. 8.

included a "notch" or downward dip. The phase angle of the emission, in contrast, typically changed slowly and smoothly with increasing stimulus levels. Note, in particular, the response for f_2 = 5 kHz in Figure 1 (right middle panel) where 3-dB steps were used.

Another typical example of the phase and amplitude changes in the echidna input–output function is shown in Figure 4, left panel pair. This is from the other ear of the animal in Figure 1. Figure 4 compares this typical growth function response with two other common methods of varying emission stimulus parameters, measured in the same ear. The middle pair of panels shows amplitude and phase responses obtained when varying only the frequency. The solid lines in the lower of the middle pair of panels indicate the slope of the phase change, which is used to calculate the emission latency. The right pair of panels shows typical responses found by varying the stimulus level ratio L_1/L_2, in this case by fixing L_1. Such frequency and level ratio measures were made in several of the animals in the study. Typical results are presented in the sections below.

Frequency ratio functions

The middle pair of panels in Figure 4 shows a typical example of the amplitude and phase response

observed when varying only f_1 for fixed and relatively low stimulus levels. There are two aspects of such responses that are of potential interest. One is the slope of the phase response, which is related to the latency of the emission. Typical results are considered in a subsequent section. The other is the shape of the amplitude response, or the "frequency ratio function," considered in this section. For the emission at $2f_1-f_2$, this is usually in the form of a "passband" response (e.g., Brown and Gaskil 1990a; Taschenberger et al. 1995; Kössl and Boyan 1998). In most animals, including birds and lizards, there is a peak in response at f_2/f_1 = 1.2–1.3. In many mammals, there is further subdividing of the response in that several sharp peaks are often observed (e.g., Mills and Rubel, 1997; Mills 2000). Typical frequency ratio functions for the echidna are shown in Figure 5 for a sequence of stimulus levels $L_1 \times L_2$ as noted. It can be seen that the echidna responses were no exception to the general rule. For most levels, there was a clear maximum response for f_2/f_1 between 1.2 and 1.3. The shaded line indicates-frequency ratios f_2/f_1 of 1.21 and 1.28. These two ratios were used to construct emission audiograms for comparison in several animals in this study.

Figure 6 shows typical emission audiograms obtained for both frequency ratios for both ears of the last animal in the study. The ABR audiogram and threshold emission audiogram for the frequency ratio f_2/f_1 = 1.21 are the same as in Figure 3, repeated for comparison. The lower, short-dashed lines show the emission audiograms for the frequency ratio f_2/f_1 = 1.28, with L_1/L_2 = 10 dB. It can be seen that the threshold results for the two frequency ratios were very similar.

For both ears in Figure 6, both emission threshold measurements gave an audiogram with a notch, or shelf, located in the region from 10 to 16 kHz. This was also seen in several other animals, but not in all (see Fig. 3.). However, the ABR responses did not show such a notch or shelf in *any* ears even though responses at appropriate stimulus frequencies were closely examined. Implications of these results are considered further in the Discussion section, in regard to the possible effects of the base cutoff frequency in the echidna.

Effects of stimulus amplitude ratio

The right panels in Figure 4 show a typical response found by varying the stimulus level L_2 while holding all other parameters constant. The case shown is for a fixed low L_1 stimulus level and for f_2 = 8 kHz. It can be seen that the amplitude response was very broad and varied slowly, with the phase angle response being similarly gentle. Typical stimulus level ratio functions for a range in L_1 are summarized in Figure 7. For

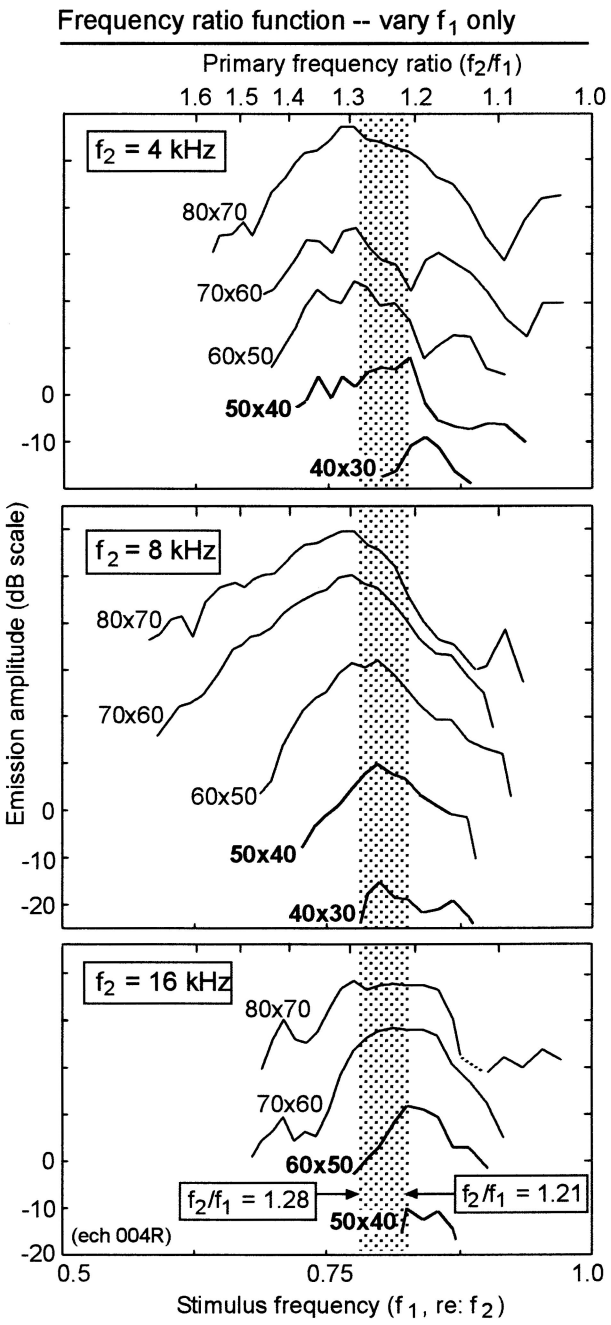


FIG. 5. Typical frequency ratio functions with stimulus level as a parameter. As in Fig. 4 (center panel), the frequency ratio function is defined by fixing the stimulus levels and the f_2 frequency and varying the f_1 frequency. The horizontal axis is the f_1 frequency, and the vertical axis is the emission amplitude. The emission amplitude (dB SPL) for the lowest curve in each panel is given on the left axis. For clarity, successive curves are shifted 10 dB vertically, otherwise they would overlap. The parameter is the stimulus level $L_1 \times L_2$, in dB SPL. For reference, the frequency ratio as it is usually presented, i.e., the ratio f_2/f_1 is listed at the top of the figure. The shaded band denotes the ratios typically used in constructing threshold curves in this study: f_2/f_1 = 1.21 was employed in all animals, while f_2/f_1 = 1.28 was included in a few cases for comparison (e.g., Fig. 6).

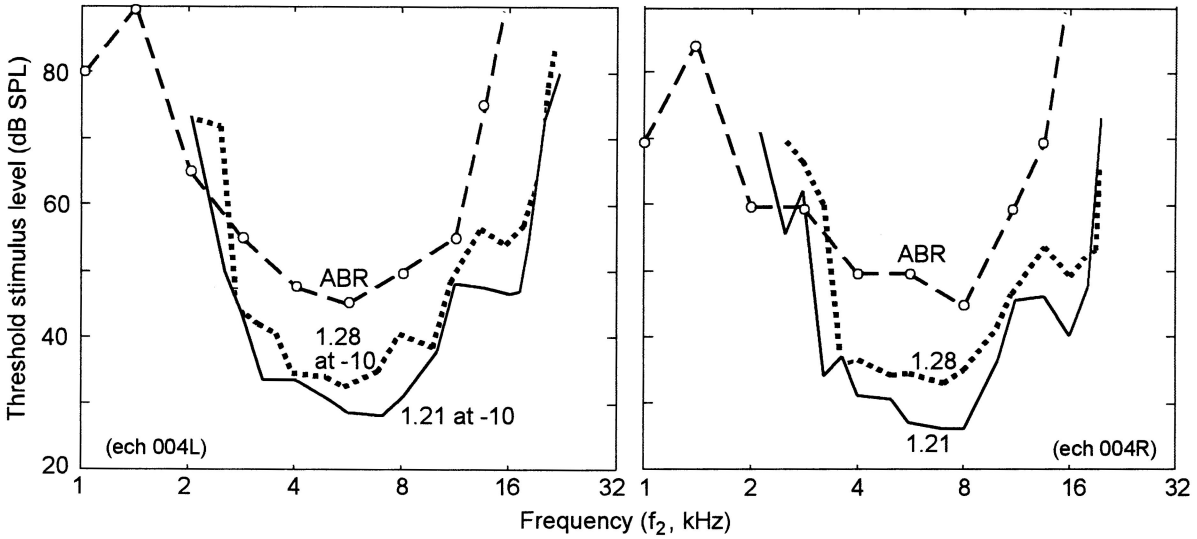


FIG. 6. Comparison in one animal (both ears) of the threshold curves as defined by emissions with stimulus frequency ratio f_2/f_1 equal to 1.21 and 1.28. The curves for $f_2/f_1 = 1.21$ (solid lines) and the ABR thresholds (open circles) are the same as in Fig. 3.

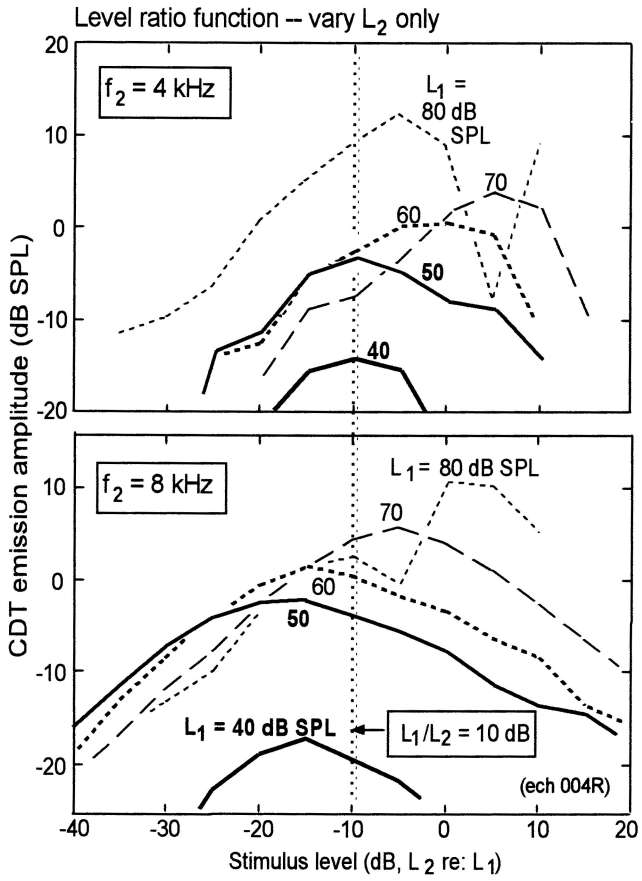


FIG. 7. Emission amplitude as a function of stimulus level ratio L_1/L_2 with stimulus frequencies fixed ($f_2/f_1 = 1.21$). Only the stimulus level L_2 was varied; the level L_1 is the parameter listed. The horizontal axis is the ratio of L_2 to L_1 in dB. The vertical axis is the emission amplitude in dB SPL. Same ear as Fig. 5.

f_2 = 4 kHz (upper panel), the responses were somewhat more rapidly varying than those at 8 kHz, but the peak was still broad. Note that at high L_1 levels, a notch in the level ratio function was seen. This is obviously the same notch seen in the input–output functions (Fig. 1) at similar levels. The dotted vertical line indicates the ratio L_1/L_2 = 10 dB, typically chosen in this study. This choice was made to obtain the highest relative emission amplitudes as a function of L_1/L_2 ratio that could be obtained at low stimulus levels over the range of f_2 frequencies important for this study. The amplitude functions observed, as illustrated in Figure 7, support this choice.

ABR and emission latencies

Typical ABR latencies for the echidna are shown in Figure 2. Note that because of the high noise floor in the echidna ABR measurements, the latencies of the first waves could not be securely detected near threshold. Therefore, the latency was quantified using the trough following Wave IV. Even so, the latencies seen in Figure 2 appeared somewhat prolonged compared with those for most therian mammals. This result is probably related to the lower body temperature of the echidna, as hypothermia is known to prolong ABR latencies in mammals (Doyle and Fria 1985; Jansen et al. 1991). Latencies in the echidna decreased with increasing stimulus level, as indicated by the dashed line in Figure 2. Typical results for the variation of latencies with stimulus level at different frequencies are given in the left panel of Figure 8. The right-hand panel summarizes the estimated latency measures at a criterion level

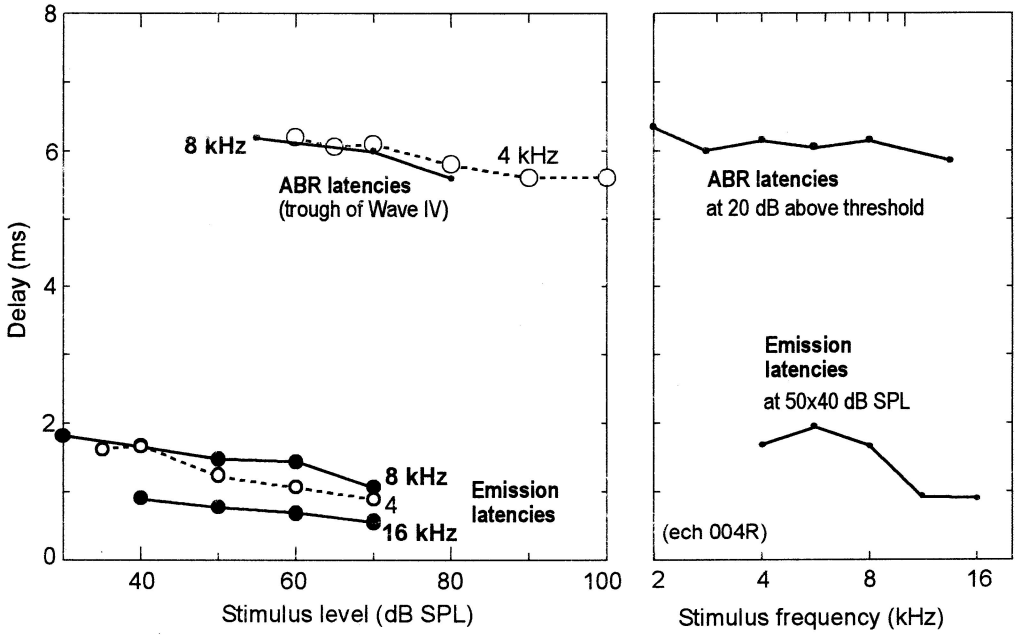


FIG. 8. Variation of latencies of emission and ABR with stimulus parameters. Emission latencies were determined for only a few ears and used a fixed-f_2 paradigm (Fig. 4, center panel). ABR latencies were measured from the negative trough of wave IV (Fig. 2). In the left panel, the stimulus level on the horizontal axis refers either to the ABR tone pip level or to the stimulus level L_2, where $L_1/L_2 = 10$ dB. The parameter is the f_2 frequency or the pip stimulus frequency as applicable. The right panel shows the latencies at given stimulus levels as a function of frequency. For the emission values, the latencies were evaluated at $L_1 \times L_2 = 50 \times 40$ dB SPL. The ABR latencies are those determined at 20 dB above threshold.

for the same ear as a function of the stimulus frequency. ABR latencies were similar for all ears tested; therefore, only a typical response is shown in Figure 8.

As noted in Figure 4, the variation of the emission phase with emission stimulus frequency ratio f_2/f_1 can also be used to obtain latency information. Of course, emission latencies reflect only cochlear processes and do not include subsequent neural transmission latencies (e.g., Brown and Kemp 1985). Because of the time required for such measurements, emission latencies were measured in only a few ears. The results for one animal are presented in Figure 8. The emission latencies obtained were comparable to those of typical therian mammals (e.g., Mills and Rubel 1997).

DISCUSSION

Relationship of ABR and emission audiograms to behavioral audiograms

In laboratory animals, tone pip ABR thresholds measured under the best conditions are typically higher than behavioral thresholds measured in the same animals. For example, the mean difference in threshold over the frequency range 0.5–4 kHz was between 10 and 15 dB, comparing evoked responses measured in the inferior colliculus of 7 chinchillas with behavioral thresholds for 0.5-s tones (Davis and Ferraro 1984, Fig. 3). More comparable to the echidna measurements, thresholds for scalp-recorded ABRs in rabbits were typically 20 dB higher than behavioral thresholds over 0.5–16 kHz (Borg and Engstrom, 1983, Fig. 7). Irrespective of the absolute threshold, the most important finding from these and similar studies is that the *shape* of the ABR threshold curve was generally a very good representation of the shape of the behavioral threshold curve. Excellent comparisons were demonstrated for normal animals and in the same animals following frequency-specific ototoxic damage.

For the echidna ABR measurements in this study, the noise level was high compared with that of common laboratory animals. This may have been a result of the relatively light anesthesia used and spontaneous activity in the large numbers of small muscles close to the surface which act to move the spines individually in this species (Allison and Goff 1972). These considerations lead us to conclude from the ABR data summarized in Figure 3 that (1) the typical echidna behavioral best threshold is *at least* as low as 20 dB SPL over 4–8 kHz and probably is considerably lower given the high noise floor in the measurements and (2) that the behavioral threshold curve for the echidna is strongly U-shaped, with the effective range of hearing being only 3 octaves (Table 1).

The behavioral sensitivity of the echidna hearing system can also be estimated by comparing the emission responses measured here to those of selected therian mammals. For the echidna, such a comparison is particularly useful because the noise floor for the echidna emission data was comparable to measurements in other animals, unlike the ABR noise floor. For the first comparison, note that the lowest behavioral thresholds of the New Zealand white rabbit are about 5–10 dB SPL, with the best thresholds occurring from 8 to 16 kHz (Martin et al. 1980). The stimulus level (L_2) to obtain an emission amplitude of −10 dB SPL in the same species was typically 30–35 dB SPL for f_2 about 8 kHz with similar stimulus ratios (i.e., f_2/f_1 = 1.25 and L_1/L_2 = 10 dB; Lonsbury–Martin et al. 1987; Whitehead et al. 1992). This is about the same or slightly higher than the levels typically required for the echidna at 8 kHz (Fig. 3; Table 1). Since this comparison is made at the one frequency where both echidna and rabbit appear to have good auditory sensitivity, i.e., 8 kHz, it seems reasonable to conclude that the auditory sensitivity of the echidna at its best threshold is probably at least as good as that of the rabbit. For a second comparison, consider the gerbil, which has good auditory sensitivity at low frequencies (Ryan 1976). A similar comparison at frequencies from 4 to 8 kHz of the emission results for the gerbil (Mills and Rubel 1996) to that for the echidna leads to a very similar conclusion, i.e., that the best behavioral threshold for the echidna is about 10 dB SPL. Note that for this comparison, the equipment, procedures, and parameter choices used for the gerbil and the echidna measurements were nearly identical.

The echidna differs from the rabbit and the gerbil in that its effective frequency range of auditory function is only 3 octaves. In general, the effective hearing range in therian mammals that are not auditory specialists and are of similar size to the echidna is between 6 and 8 octaves (Echteler et al. 1994, Table 5.1). By all measures, then, the range of effective hearing in the echidna appears to be significantly narrower than that of comparable therian mammals. However, the echidna absolute best thresholds appear to be typical of other mammals that are not auditory specialists, e.g., those that are not predators which depend on their hearing, such as canines and felines.

In spite of its short range, however, the echidna upper limit of hearing appears to extend to somewhat higher frequencies than does that of *any* of the birds or reptiles (reviews: Fay 1988, 1994; Manley 1990, 2000; Dooling et al. 2000). Even for an avian auditory specialist like the barn owl, the effective upper range of hearing is only 11–12 kHz and its threshold curve increases very steeply at these frequencies (Konishi 1973). The echidna upper limit of effective hearing is estimated to be about 16 kHz in comparison, and function clearly extends above 20 kHz (Figs. 1, 3, and 6, and Table 1).

Our findings also should be compared with the only previously published results of measurements of the echidna peripheral auditory system. On the basis of measurements of the echidna middle ear, Aitkin and Johnstone (1972) suggested that the echidna had a relatively stiff, "primitive" middle ear that conducted sound over a narrow frequency range and that absolute auditory sensitivity was probably poor. However, current analysis suggests that the echidna middle ear does not represent an example of the primitive condition for mammals but a derived form common to extant rodents and many other mammals (Rosowski 1992, 1994). In particular, the echidna exhibits a large, obvious orbicular apophysis of the malleus (Griffiths 1968). This bony mass, called the "head" of the malleus in humans, is thought to represent a derived trait that evolved to provide an additional "high-frequency" transmission mode through the modern mammalian middle ear (Fleischer 1978). In summary, the results here for the echidna auditory function are generally compatible with the relatively narrow frequency range suggested by the earlier middle ear measurements but are not in agreement with the poor absolute auditory system sensitivity suggested by Aitkin and Johnstone (1972).

Base cutoff frequency in the echidna

Most (4 of 6) of the emission audiograms showed a "notch" or "shelf" at about 12–16 kHz (Fig. 3). However, none of the ABR threshold audiograms showed such a notch even though the appropriate stimulus frequencies were closely examined. Therefore, such a notch is unlikely to represent an actual enhancement of auditory sensitivity in this narrow frequency range. However, when the notch occurred in an animal's ear, it was seen persistently (Fig. 6). The persistence suggests that the notch was not just a minor phenomenon resulting from phase cancellation at a given frequency or a similar effect. Earlier work suggests that such notches might be due to a phenomenon unique to two-tone emission measurements (Mills 1997; Mills and Rubel 1998). Model studies of the therian cochlear amplifier show that such notch behavior occurs naturally as the two stimulus frequencies approach the frequency above which traveling waves cannot propagate basalward down the basilar membrane. As the stimulus frequencies approach the cutoff frequency from below, the interaction between the two stimuli results in modest increases in the measured emission amplitude and in the apparent cochlear amplifier gain. This results in a relative decrease in the "threshold" in the emission audiogram over a short frequency interval near the upper limit of hearing. That is, the absolute

CDT amplitude and apparent gain reach a relative maximum at the "peak frequency," above which both decrease sharply. Such effects have previously been detailed in measurements in gerbils (Mills and Rubel 1998), where the variation in apparent cochlear amplifier gain was used to investigate the development of the base cutoff frequency with age. The apparent peak in the measured cochlear amplifier gain in the adult gerbil occurred at 42 kHz. The comparable peak frequency for the echidna is approximately the typical frequency at the upper end of the notch, i.e., about 16 kHz (Figs. 3 and 6). This is 1.3 octaves below that in the gerbil.

In sum, the notch in the emission data suggests that the echidna has a distribution of passive resonance along its basilar membrane and a similar relationship of passive to active cochlear response as seen in other mammals. However, the resonance frequency at the base of the cochlea is estimated to be only 16 kHz for the echidna. This is similar to that in human and chimpanzee but much lower than for almost all other therian mammals. Also note that the results of the gerbil observations and model studies suggest that only if the cochlear amplifier is functioning normally at the base of the cochlea and if the middle ear is functional across these frequencies, will such a notch appear (Mills 1997; Mills and Rubel 1998). If these two situations do not occur, such as in typical cases of modest hearing loss of cochlear origin where the function of the cochlear amplifier deteriorates smoothly as frequency increases, the emission audiogram would rise smoothly without a notch being obvious. This could explain why some echidna ears did not show the notch (Fig. 3). It is also important to note that, to date, the effects of the base cutoff frequency on two-tone emissions have been modeled only for the cochlear amplifier type posited for therian mammals (Mills 1997). It is not known if similar effects would occur for other types of cochlear amplification. Therefore, the fact that the echidna emission responses do show such behavior only suggests that the echidna cochlear amplifier *could be* of the same type as that of therian mammals.

Parametric characteristics of monotreme emissions

Distortion product otoacoustic emissions are observed from nearly all vertebrate ears and even from insects (e.g., Zwicker 1981; Brown 1987; Lonsbury–Martin et al. 1987; Norton and Rubel 1990; Johnstone et al. 1990; Kössl 1992; Manley et al. 1993; Taschenberger et al. 1995; Faulstich et al. 1996; Kössl and Boyan 1998; Taschenberger and Manley 1998). There is a wide variation in the characteristics of the observed emissions, including a strong but generally poorly characterized dependence on the stimulus parameters. Across classes, only emission growth functions have typically been reported, usually at different, idiosyncratic, choices of parameters. A recent study of frequency ratio functions in birds and lizards (Taschenberger et al. 1995) is a useful first step in the required characterization. However, lacking exhaustive studies in all vertebrate classes over the complete four-dimensional stimulus parameter space (i.e., f_1, f_2, L_1, L_2: Whitehead et al. 1992; Mills and Rubel 1994), it cannot be claimed that the monotreme emission characteristics, or even those of therian mammals, are categorically similar to or different than those of any other vertebrate class.

The remaining discussion focuses on comparison of emissions from echidna with those from therian mammals, of which there are a few species that have been studied over an adequate variation in stimulus parameters, including gerbils (Mills and Rubel 1994), guinea pigs (Brown and Gaskill 1990b), and rabbits (Whitehead et al. 1992). Because of time constraints, echidna emissions could not be characterized to the extent that these laboratory animals have been. The echidna data set includes growth functions at $f_2/f_1 = 1.21$ and $L_1/L_2 = 10$ dB over the complete range of f_2 frequencies for every ear studied (e.g., Fig. 1) plus a similar set of growth functions for $f_2/f_1 = 1.28$ in two ears (not shown, but see the resulting threshold curves in Fig. 6). These growth functions were supplemented in several animals by frequency ratio functions as illustrated in Figure 4 (center panel) and Figure 5, and by level ratio functions such as those in Figure 4 (right panel) and Figure 7. These are incomplete characterizations, of course, as these functions were obtained only for a limited range of the other parameters.

Within this limited data set, the echidna emissions are found to be generally similar to those of the therian mammals noted. The echidna growth functions, for example, were seen to typically rise steeply from the noise floor at relatively low stimulus levels, reach a "saturation" region, including a modest notch or decrease in amplitude, and then, at very high stimulus levels, resume a sharp upward course. The saturation region typically is quite extended in the echidna, i.e., it occurs over a wide stimulus range (Figs. 1 and 4). In fact, the saturation region frequently appears to be more extended than those in the laboratory animals listed.

The phase angle responses observed in the echidna growth functions clearly differ from those usually found in gerbil and rabbit. Consider the "notch" often seen in these input–output growth functions (e.g., Lonsbury–Martin et al. 1987; Whitehead et al. 1992; Mills and Rubel 1994; Mills 1997). A sharp notch is usually associated with an abrupt 180° shift in the emission phase angle. Model studies show that this behavior

can be explained as the result of summing emissions coming from slightly different regions of the cochlea, when the intrinsic phase angles of the two regions differ by about 180° (Mills 1997). While the echidna growth functions often show a modest notch in amplitude, it is not as sharp as seen in gerbil and rabbit. Also, a typical abrupt change in phase angle as the stimulus level increases through this notch region is not seen. Rather, there is usually a very smooth increase in phase angle as the transition is made from the "active" emission response to the "passive" response (Figs. 1 and 4).

In summary, the parametric emission results for the echidna show many similarities to typical responses in therian mammals, but they also show intriguing differences. A detailed comparison to other vertebrate classes has not been presented because of a general lack of results for comparable parameters.

Evolution of the cochlear amplifier

To provide amplification of the sound energy at kHz frequencies in the inner ear, two mechanisms have been proposed. (1) In therian mammals, it has been suggested that the force generator originates in the electromotility of the outer hair cells (OHCs), i.e., the change in OHC length due to a change in OHC membrane voltage (e.g., Ashmore 1987; Zheng 2000; reviews: Dallos 1992; Manley and Köppl 1998). While the ubiquity of this phenomenon in therian mammals seems established, as well as its nonexistence in inner hair cells and in all hair cell types in nonmammals, so also is the difficulty that the OHC membrane capacitance limits its high frequency response (e.g., Santos–Sacchi 1992). It seems extremely difficult to explain how this mechanism could work at 100 kHz as required for a bat or marine mammal. It might be that while OHC electromotility is certainly related to cochlear amplifier function in therian mammals, it is not the actual mechanism that provides cycle-by-cycle power input to the traveling wave, at least at high frequencies. (2) In birds and reptiles, it has been suggested that the amplification is due to a calcium-dependent process operating at the site of the transduction channel (e.g., Eguiluz et al 2000; Martin et al. 2000; reviews: Hudspeth 1997; Manley and Köppl 1998; Gleich and Manley 2000). The possibility has been raised that a similar process may act in mammalian OHCs as well, although there is admittedly little evidence for this hypothesis at present.

While the possible contribution of such calcium-dependent processes remains unclear and untestable, at least it could be determined if monotreme OHCs possessed electromotility like therian mammals. If so, and if the same motor protein were found responsible (Zheng et al. 2000), it would suggest strongly that electromotility was a primitive condition for mammals. This would imply that electromotility could be the central mechanism for the mammalian cochlear amplifier. Such a discovery would suggest that electromotility would have been present in the last common ancestor of monotremes and therian mammals, i.e., in the stem mammal-like reptiles that lived about 180 million years ago. It would be equally important if monotreme OHCs were found *not* to possess electromotility. Since monotremes do have a cochlear amplifier of some kind and an organ of Corti, this finding would suggest that some other mechanism provided amplification. This would be particularly interesting because this unknown mechanism would have to provide effective amplification at frequencies over 20 kHz (Figs. 1, 3, and 6). This unknown mechanism (1) could be a derived trait in the monotreme order, i.e., it developed independently in the monotreme line after the divergence of monotremes from mainstream therian mammals; (2) could be a primitive trait in mammalian evolution, in which case it could be still present in therian mammals and could be the actual amplification mechanism of the cochlear amplifier in all modern mammals; or (3) if this same mechanism were also found to be present in birds and reptiles, it would suggest that it had evolved even earlier, e.g., in the stem tetrapods leading to mammals, birds, and reptiles, and could then be a common mechanism, providing amplification in all these vertebrate classes.

In sum, the results presented in this article show that the echidna has a high-frequency limit to its hearing response that is midway between typical therian mammals on the one hand and birds and reptiles on the other. The emission measurements particularly suggest that monotremes could have a cochlear amplifier similar to that in therian mammals, with similar sensitivity but with a significantly shorter frequency range. However, the possibility that the monotremes utilize an amplification process different than in therian mammals cannot be ruled out on current evidence. The echidna auditory response and other characteristics of the monotreme auditory system do have intriguing differences from therian mammals and similarities to those of extant birds and reptiles. Because of their unique position among vertebrates, monotremes offer unique opportunities for further study of the function and evolution of cochlear mechanism.

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REFERENCES

AITKIN L. The auditory neorobiology of marsupials: A review. Hear. Res. 82:257–266, 1995.

AITKIN L. Hearing, the Brain, and Auditory Communication in Marsupials. Springer–Verlag, New York, 1998.

AITKIN L, NELSON J, SHEPHERD R. Development of hearing and vocalization in a marsupial, the Northern Quoll, *Dasyurus hallucatus*. J. Exp. Zool. 276:394–402, 1996.

AITKIN LM, JOHNSTONE BM. Middle-ear function in a monotreme: The echidna (*Tachyglossus aculeatus*). J. Exp. Zool. 180:245–250, 1972.

ALLIN EF. The auditory apparatus of advanced mammal-like reptiles and early mammals. In: Holton N, MacLean PD, Roth JJ, Roth EC, (eds) The Ecology and Biology of Mammal-like Reptiles. Smithsonian Institution Press, Washington, DC 1986, pp 283–294.

ALLISON T, GOFF WR. Electrophsiological studies of the echidna, *Tachyglossus aculeatus*. III. Sensory and interhemispheric evoked responses. Arch. Ital. Biol. 110:195–216, 1972.

ASHMORE JF. A fast motile response in guinea-pig outer hair cells: The cellular basis of the cochlear amplifier. J. Physiol. (Lond.) 388:323–347, 1987.

AUGEE M, GOODEN B. Echidnas of Australia and New Guinea. New South Wales University Press, Kensington, NSW, Australia, 1993.

BORG E, ENGSTROM B. Hearing thresholds in the rabbit. Acta Otolaryngol. 95:19–26, 1983.

BROWN AM. High frequency peaks in the cochlear microphonic response of rodents. J. Comp. Physiol. 83:377–392, 1973.

BROWN AM. Acoustic distortion from rodent ears: A comparison of responses from rats, guinea pigs and gerbils. Hear. Res. 31:25–38, 1987.

BROWN AM, GASKILL SA. Can basilar membrane tuning be inferred from distortion measurement? In: Dallos P, Geisler CD, Mathews JW, Ruggero MA, Steele CR, (eds) The Mechanics and Biophysics of Hearing: Proceedings of a Conference held at the University of Wisconsin, Madison, WI, June 25–29, 1990. Springer-Verlag, New York, 1990a, pp 164–169.

BROWN AM, GASKILL SA. Measurement of acoustic distortion reveals underlying similarities between human and rodent mechanical responses. J. Acoust. Soc. Am. 88:840–849, 1990b.

BROWN AM, KEMP DT. Intermodulation distortion in the cochlea: could basal vibration be the major cause of round window CM distortion? Hear Res. 19:191–198, 1985.

BROWN AM, MCDOWELL B, FORGE A. Acoustic distortion products can be used to monitor the effects of chronic gentamicin treatment. Hear. Res. 42:143–156, 1989.

BROWN MC, SMITH DI, NULTALL AL. The temperature dependency of neural and hair cell responses evoked by high frequencies. J. Acoust. Soc. Am. 73:1662–1670, 1983.

CARROLL RL. Vertebrate Paleontology and Evolution. Freeman, New York, 1988.

CHEN CS, ANDERSON LM. The inner ear structures of the echidna—an SEM study. Experientia 41:1324–1326, 1985.

CODY AR. Acoustic lesions in the mammalian cochlea: Implications for the spatial distribution of the 'active process.' Hear. Res 62:166–172, 1992.

CONE–WESSON BK, HILL KG, LIU G-B. Auditory brainstem response in tammar wallaby (*Macropus eugenii*). Hear. Res. 105:119–129, 1997.

CORWIN JT, BULLOCK TH, SCHWEITZER K. The auditory brain stem response in five vertebrate classes. Electroenchephalogr. Clin. Neurophysiol. 54:629–641, 1982.

DALLOS P. The active cochlea. J. Neurosci. 12:4575–4585, 1992.

DAVIS H. An active process in cochlear mechanics. Hear. Res. 9:79–90, 1983.

DAVIS RI, FERRARO JA. Comparison between AER and behavioral thresholds in normally and abnormally hearing chinchillas. Ear Hear. 5:153–159, 1984.

DOOLING RJ, FAY RR, POPPER AN. Comparative Hearing: Birds and Reptiles (Springer Handbook of Auditory Research, Vol. 13) Springer–Verlag, New York, 2000.

DOYLE WJ, FRIA TJ. The effects of hypothermia on the latencies of the auditory brain-stem response (ABR) in the rhesus monkey. Electroenchephalogr. Clin. Neurophysiol. 60:258–266, 1985.

ECHTELER SM, FAY RR, POPPER AN. Structure of the mammalian cochlea. In: Popper AN, Fay RR, (eds) Comparative Hearing: Mammals (Springer Handbook of Auditory Research, Vol. 4). Springer–Verlag, New York, 1994, 134–171.

EQUILUZ VM, OSPECK M, CHOE Y, HUDSPETH AJ, MAGNASCO MO. Essential nonlinearities in hearing. Phys. Rev. Lett. 84:5232–5235, 2000.

FAULSTICH M, KÖSSL M, REINER K. Analysis of non-linear cochlear mechanics in the marsupial *Monodelphis domestica*: ancestral and modern mammalian features. Hear Res. 94:47–53, 1996.

FAY RR. Hearing in Vertebrates: A Psychophysics Databook. Hill-Fay Associates, Winetka, IL, 1988.

FAY RR. Comparative auditory research. Popper AN, Fay RR, Comparative Hearing: Mammals (Springer Handbook of Auditory Research, Vol. 4) Springer–Verlag, New York, 1994, pp 1–17.

FLEISCHER G. Evolutionary principles of the mammalian middle ear. Adv. Anat. Embryol. Cell Biol. 55:1–67, 1978.

FOX RC, MENG J. An X-radiographic and SEM study of the osseous inner ear of multituberculates and monotremes (Mammalia): implications for mammalian phylogeny and evolution of hearing. Zool. J. Linnean Soc. 121:249–291, 1997.

GATES GR, SAUNDERS JC, BOCK GR. Peripheral auditory function in the platypus, *Ornithorthynchus anatinus*. J. Acoust. Soc. Am. 56:152–156, 1974.

GLEICH O, MANLEY GA. The hearing organ of birds and Crocodilia. In: Dooling RJ, Fay RR, Popper AN, (eds) Comparative Hearing: Birds and Reptiles (Springer Handbook of Auditory Research, Vol. 13) Springer–Verlag, New York, 2000, pp 70–138.

GRANT T. The Platypus: A Unique Mammal. University of New South Wales Press Sydney, Australia, 1995.

GRIFFITHS M. ECHIDNAS. Pergamon, London, 1968.

GRIFFITHS M. The Biology of the Monotremes. Academic Press, New York, 1978.

GRIGG GC, AUGEE ML, BEARD LA. Thermal relations of free-living echidnas during activity and in hibernation in a cold climate. In: Augee ML, (ed) Platypus and Echidnas. Royal Zoology Society of NSW, Mosmon, NSW, Australia, 1992, pp 160–173.

GUMMER AW, SMOLDERS JWT, KLINKE R. Mechanics of a single-ossicle ear: I. The extrastapedius of the pigeon. Hear. Res. 39:1–14, 1989a.

GUMMER AW, SMOLDERS JWT, KLINKE R. Mechanics of a single-ossicle ear: II. The columelia footplate of the pigeon. Hear. Res. 39:15–26, 1989b.

HU Y, WANG Y, LUO Z, LI C. A new symmetrodont mammal from China and its implications for mammalian evolution. Nature 390:137–142, 1997.

HUDSPETH AJ. Mechanical amplification of stimuli by hair cells. Curr. Opin. Nerobiol. 7:480–486, 1997.

JANSSEN R, HETZLER BE, CREASON JP, DYER RS. Differential impact of hypothemia and pentobarbital on brain-stem auditory evoked responses. Electroenchephalogr. Clin. Neurophysiol. 80:412–421, 1991.

JOHNSTONE BM, GLEICH B, MAVADAT N, MCALPINE D, KAPADIA S. Some properties of the cubic distortion tone emission in the guinea pig. Adv. Audiol. 7:57–62, 1995.

JØRGENSEN JM, LOCKET NA. The inner ear of the echidna *Tachyglossus aculeatus*: the vestibular sensory organs. Proc. R. Soc. Lond. B 260:183–189, 1995.

KEMP DT. Stimulated acoustic emissions from within the human auditory system. J. Acoust. Soc. Am. 64:1386–1391, 1978.

KONISHI M. How the owl tracks its prey. Am. Sci. 61:414–424, 1973.

KÖSSL M. High frequency distortion products from the ears of two bat species, *Magaderma Iyra* and *Corollia perspicillata*. Hear. Res. 60:156–164, 1992.

KÖSSL M, BOYAN GS. Acoustic distortion products from the ear of a grasshopper. J. Acoust. Soc. Am. 104:326–335, 1998.

KÖSSL M, VATER M. Cochlear structure and function in bats. Popper AN, Fay RR, Hearing by Bats (Springer Handbook of Auditory Research, Vol. 5). Springer–Verlag, New York, 1995, 191–234.

LADHAMS A, PICKLES JO. Morphology of the monotreme organ of Corti and the macula lagena. J. Comp. Neurol. 366:335–347, 1996.

LIBERMAN MC. The cochlear frequency map for the cat: labeling auditory nerve fibers of known characteristic frequency. J. Acoust. Soc. Am. 72:1441–1449, 1982.

LONSBURY–MARTIN BL, MARTIN GK, PROBST R, COATS AC. Acoustic distortion products in rabbit ear canal. I. Basic features and physiological vulnerability. Hear. Res. 28:173–189, 1987.

LUO Z, CROMPTON AW, LUCAS SG Evolutionary origin of the mammalian promontorium and cochlea. J. Vertebr. Paleontol. 15:113–121, 1995.

LUO Z, KETTEN DR. CT scanning and computerized reconstruction of the inner ear of multituberculate mammals. J. Vertebr. Paleontol. 11:220–228, 1991.

MANLEY GA. The middle ear of the Tokay gecko. J. Comp. Physiol. 81:239–250, 1972a.

MANLEY GA. Frequency response of the middle ear of geckos. J. Comp. Physiol. 81:251–258, 1972b.

MANLEY GA. Peripheral Hearing Mechanisms in Birds and Reptiles. Springer–Verlag, New York, 1990.

MANLEY GA. Cochlear mechanisms from a phylogenetic viewpoint. Proc. Natl. Acad. Sci. USA 97:11736–11743, 2000.

MANLEY GA, KÖPPL C. Phylogenetic development of the cochlea and its innervation. Curr. Opin. Neurobiol. 8:468–474. 1998.

MANLEY GA, KÖPPL C, JOHNSTONE BM. Distortion-product otoacoustic emissions in the bobtail lizard. I: General characteristics. J. Acoust. Soc. Am. 93:2820–2833, 1993.

MARTIN GK, LONSBURRY–MARTIN BL, KIMM J. A rabbit preparation for neurobehavioral auditory research. Hear. Res. 2:65–78, 1980.

MARTIN P, MEHTA AD, HUDSPETH AJ. Negative hair bundle stiffness betrays a mechanism for mechanical amplification by the hair cell. Proc. Natl. Acad. Sci. USA 97:12026–12031, 2000.

MESSER M, WEISS AS, SHAW DC, WESTERMAN M. Evolution of the monotremes: Phylogenetic relationship to marsupials and eutherians, and estimation of divergence dates based on α-lactalbumin amino acid sequences. J. Mammalian Evol. 5:95–105, 1998.

MILLS DM. Interpretation of distortion product otoacoustic emission measurements. I. Two stimulus tones. J. Acoust. Soc. Am. 102:413–429, 1997.

MILLS DM. Frequency responses of two- and three-tone distortion product otoacoustic emissions in Mongolian gerbil. J. Acoust. Soc. Am. 107:2586–2602, 2000.

MILLS DM, RUBEL EW. Variation of distortion product otoacoustic emissions with furosemide injection. Hear. Res. 77:183–199, 1994.

MILLS DM, RUBEL EW. Development of the cochlear amplifier. J. Acoust. Soc. Am. 100:428–441, 1996.

MILLS DM, RUBEL EW. Development of distortion product emissions in the gerbil: "filter" response and signal delay. J. Acoust. Soc. Am. 101:395–411, 1997.

MILLS DM, RUBEL EW. Development of the base of the cochlea: Place code shift in the gerbil. Hear. Res. 122:82–96, 1998.

NORTON SJ, RUBEL EW. Active and passive ADP components in mammalian and avian ears. In: Dallos P, Geisler CD, Mathews JW, Ruggero MA, Steele CR, (eds) The Mechanics and Biophysics of Hearing. Springer–Verlag, New York, 1990, pp 219–227.

PATUZZI R, ROBERTSON D. Tuning in the mammalian cochlea. Physiol. Rev. 68:1009–1082, 1988.

PICKLES JO. An introduction to the Physiology of Hearing. Academic Press San Diego, CA, 1988.

POPPER AN, FAY RR, (eds). Hearing by Bats (Springer Handbook of Auditory Research, Vol. 5) Springer–Verlag, New York, 1995.

POPPER AN, FAY RR. The auditory periphery in fishes. In: Fay RR, Popper AN, (eds) Comparative Hearing: Fish and Amphibians (Springer Handbook of Auditory Research, Vol. 11). Springer–Verlag, New York, 1999, pp 43–100.

REIMER K. Hearing in the marsupial *Monodephis domestica* as determined by auditory-evoked brainstem responses. Audiology 34:334–342, 1995.

RHODE WS, RECIO A. Study of mechanical motions in the basal region of the chinchilla cochlea. J. Acoust. Soc. Am. 107:3317–3332, 2000.

ROSOWSKI JJ. Hearing in transitional mammals: Predictions from the middle-ear anatomy and hearing capabilities of extant mammals. In: Webster DB, Fay RR, Popper AN, (eds) The Evolutionary Biology of Hearing. Springer–Verlag, New York, 1992, pp 615–631.

ROSOWSKI J. Outer and middle ears Popper AN, Fay RR, Comparative Hearing: Mammals (Springer Handbook of Auditory Research, Vol. 4). Springer–Verlag, New York, 1994, pp 172–247.

RUGGERO MA, RICH NC. Furosemide alters organ of Corti mechanics: Evidence for feedback of outer hair cells upon the basilar membrane. J. Neurosci. 11:1057–1067, 1991.

RUSSELL IJ, NILSON KE. The location of the cochlear amplifier: Spatial representation of a single tone on the guinea pig basilar membrane. Proc. Natl. Acad. Sci. USA 94:2660–2664, 1997.

RYAN A. Hearing sensitivity of the mongolian gerbil, *Meriones unguiculatis*. J. Acoust. Soc. Am. 59:1222–1226, 1976.

SANTOS-SACCHI J. On the frequency limit and phase of outer hair cell motility: Effects of the membrane filter. J. Neurosci. 12:1908–1916, 1992.

SAUNDERS JC, DUNCAN RK, DOAN DE, WERNER YL. The middle ear of reptiles and birds. In: Dooling RJ, Fay RR, Popper AN, (eds) Comparative Hearing: Birds and Reptiles (Springer Handbook of Auditory Research, Vol. 13). Springer–Verlag, New York, 2000, 13–69.

SELLICK PM, PATUZZI R, JOHNSTONE BM. Measurement of basilar membrane motion in the guinea pig using the Mossbauer technique. J. Acoust. Soc. Am. 72:131–141, 1982.

SHEPHERD RK, MARTIN RL. Onset of ototoxicity in the cat is related to onset of auditory function. Hear. Res. 92:131–142, 1995.

TASCHENBERGER G, GALLO L, MANLEY GA. Filtering of distortion-product otoacoustic emissions in the inner ear of birds and lizards. Hear. Res. 91:87–92, 1995.

TASCHENBERGER G, MANLEY GA. General characteristics and suppression tuning properties of the distortion-product otoacoustic emission $2f_1-f_2$ in the barn owl. Hear. Res. 123:183–200, 1998.

von BÉKÉSY G. Experiments in Hearing. McGraw–Hill, New York, 1960.

WEBSTER DB, FAY RR, POPPER AN. The Evolutionary Biology of Hearing. Springer–Verlag, New York, 1992.

WHITEHEAD ML, LONSBURY–MARTIN BL, MARTIN GK. Evidence for two discrete sources of $2f_1-f_2$ distortion-product otoacoustic emission in rabbit. I: Differential dependence on stimulus parameters. J. Acoust. Soc. Am. 91:1587–1607, 1992.

ZHENG J, SHEN W, HE DZZ, LONG KB, MADISON LD, DALLOS P. Prestin is the motor protein of cochlear outer hair cells. Nature 405:149–155, 2000.

ZWICKER E. Dependence of level and phase of the $(2f_1-f_2)$-cancellation tone on frequency range, frequency difference, level of primaries, and subject. J. Acoust. Soc. Am. 70:1277–1288, 1981.

LUO Z, CIFELLI RL, KIELAN-JAWOROWASKA Z. Dual origin of tribosphenic mammals. Nature 409:53–57, 2001.