Development of distortion product emissions in the gerbil: "Filter" response and signal delay

David M. Mills[a] and Edwin W Rubel
Virginia Merrill Bloedel Hearing Research Center, University of Washington, Seattle, Washington 98195

(Received 3 April 1996; revised 20 August 1996; accepted 10 September 1996)

Amplitude and phase responses of distortion product otoacoustic emissions as a function of stimulus frequency ratio were measured for frequencies between 2 and 48 kHz, in Mongolian gerbils (*Meriones unguiculates*) aged 15 to 30 days after birth. After baseline measurements, furosemide was administered to distinguish active from passive emissions. At all ages, structure in the form of multiple peaks was observed in the amplitude responses of specific odd-order emissions. This structure depended on the *emission* frequency, not the stimulus frequency ratio, and did not generally depend on the stimulus amplitude. Nor was it dependent on the functioning of the cochlear amplifier: At moderate stimulus levels, the observed emission distribution simply shifted to lower amplitudes when the cochlear amplifier was made temporarily dysfunctional by furosemide injection. The center frequencies and widths of the peaks in the amplitude response did not generally change with age, except that the relative amplitudes of the higher-frequency peaks were increased in younger animals. At 2 kHz, however, the distribution showed other evidence of maturation, with the frequency of maximum emission moving downward with age. The phase responses yielded estimates of the round trip signal (group or traveling wave) delay. At a given frequency, the active signal delay typically decreased substantially with increasing stimulus level. However, there was a rapid variation in delay as the stimulus level passed the normal active–passive crossover level. At stimulus levels measured *relative* to the active–passive crossover level, i.e., either 20 or 30 dB lower, the active signal delay decreased only slightly with age. Overall, both filter response and signal delay characteristics were found to be essentially mature near the onset of hearing. [S0001-4966(97)04601-8]

PACS numbers: 43.64.Jb, 43.64.Kc [RDF]

INTRODUCTION

The variation of distortion product emissions as a function of stimulus frequency ratio has been important in the investigation of cochlear mechanics. There have been two main applications, one associated with the interpretation of the amplitude response of the emissions, the other with the interpretation of the phase angle response. Both applications have obvious relevance to the study of the mechanics in the developing cochlea.

It is well known that there is structure in the amplitude response of specific odd-order emissions when the stimulus frequency ratio is varied (Brown and Gaskill, 1990b; Gaskill and Brown, 1990). This structure appears to be associated with the *emission* frequency, not with the stimulus frequency ratio itself (Brown and Gaskill, 1990a). That is, for constant stimulus levels the amplitudes of these emissions appear to peak when the emission frequency is about one half-octave below the higher-frequency stimulus (Brown and Gaskill, 1990b; Brown *et al.*, 1992). This discovery has been used to support the idea that there is a ''second filter'' in the cochlea, which filters the emissions *after* generation (Brown and Williams, 1993). Specific micromechanical structures have been proposed to account for the filtering (Allen and Fahey, 1993a, b).

It seems useful to extend these investigations into the developing mammalian cochlea, and to more completely characterize the emission amplitude response as a function of stimulus amplitude, frequency, and age of the animal. If the filtering is due to specific micromechanical structures, the characteristics of this filtering may change as these structures develop. Overall, emission amplitude responses should be related to developmental changes in the interaction of waves of different frequencies in the cochlea. These interactions must depend, for example, on the extent of the region of active amplification along the basilar membrane (BM), and on the sharpness of the peak BM response.

Separate from the amplitude response, the phase angle response has primarily been employed as a measure of the round trip travel time. This time has been variously called the group, signal, or traveling wave delay (Brown and Kemp, 1985; Kimberley *et al.*, 1993; Plonsey and Collin, 1961). Contrasted with the phase velocity, the signal velocity is the speed associated with energy or information transmission in a medium (Plonsey and Collin, 1961). For distortion product emission measurements, the signal delay specifically is the round trip time associated with a stimulus signal passing the microphone and going into the cochlea, traveling down the BM, generating an emission, and the emission traveling back out of the cochlea and being detected by the microphone. With certain assumptions, the round trip signal delay time can be related to the measured change in phase in the emission at the microphone location relative to that of the stimu-

[a]Electronic mail: dmmills@u.washington.edu

lus, the phase changes occurring when the ratio of the stimulus frequencies is changed.

The major difficulty with the interpretation of the signal delay as measured by emissions is the fact that the derived signal delays depend strongly on stimulus level, typically decreasing with increasing stimulus level (Brown and Kemp, 1985). It is difficult to know how to relate the wide span in measured values to physical distances in the cochlea. It has been suggested that the *differences* between signal delays at the same stimulus intensities but at different frequencies represent a valid comparison between traveling wave delays at different frequencies (Kimberley *et al.*, 1993). However, the situation cannot be resolved so easily. Because of possible differences in passive conduction into the cochlea at different frequencies and ages, the equivalent stimulus levels in the cochlea at different frequencies cannot be *assumed* to occur with equal stimulus levels in the ear canal. There is, after all, no *a priori* reason to chose ''equal'' stimulus levels at any particular point and using any particular measure. That is, the quantity to be ''equalized'' at different frequencies or ages could equally well be stimulus pressure, displacement, volume velocity, or energy flow (Keefe *et al.*, 1993), and the point of equalization could be any point along the input transmission path to the cochlea.

This is a particularly vexing problem in interpreting changes in signal delay during development, given the known changes in passive conductance into the mammalian cochlea during development (Mills *et al.*, 1994; Mills and Rubel, 1996). For example, there have been two recent measurements of the signal delays in humans, comparing term infants and adults (A. M. Brown *et al.*, 1994; D. Brown *et al.*, 1994). Both measured delays at about the same, constant stimulus pressure levels in the ear canal. At mid-frequencies, the first study found a decrease in the mean delay with age and the second study an increase; the reasons for the difference are not known.

At this point, it seems important to establish valid procedures for the measurement of delays using experimental animals; for these animals distortion product emissions are much easier to measure than for human infants and the results can be compared to independent measures of cochlear travel time.

I. METHODS

A. Animal preparation

Young gerbils (*Meriones unguiculates*) were obtained from breeding pairs maintained in our colony, originally purchased from a commercial supplier (Tumblebrook Farms, Brookfield, MA). Pairs were checked for births daily, and the date the birth was first observed was denoted 0 days after birth (dab). All animal preparation and recording were performed in an I.A.C. double walled acoustic booth. Animals were initially anesthetized with a subcutaneous injection of a mixture of ketamine hydrochloride (Ketaset: 15 mg/kg) and xylazine (Rompun: 5 mg/kg). A surgical depth of anesthesia was maintained by subsequent injections, as needed, of either ketamine alone or the ketamine-xylazine mixture, at about one half the initial dosage. The pinna, surrounding skin, and outer third of the ear canal were removed on the left side, along with much of the scalp. The skull was attached to a head holder with cryanoacrylic adhesive (Borden), and a thermocouple placed in the rectum. An automatic heating pad kept the internal temperature at 36–37 °C. Tissue was removed over the bulla immediately posterior to the ear canal, and a hole (1–2 mm diameter) drilled into the bulla to equalize static pressure in the outer and middle ears. The bulla hole was left open, to improve low-frequency sound conduction through the middle ear in these young animals (Cohen *et al.*, 1993; Mills *et al.*, 1994).

B. Equipment and procedures

The equipment and basic procedures employed for the high-frequency distortion product measurements were the same as previously reported (Mills and Rubel, 1996). Briefly, a coupler was employed which incorporated two microphones and two sound delivery tubes connected to a 2-mm diameter central cavity. One microphone was a 1/4-in. high-frequency microphone (Larson & Davis 2530) calibrated as a probe microphone, with the probe tip located at the coupler opening. The other was a removable ER-10B low noise microphone (Etymotic). Using a micromanipulator and operating microscope, the coupler was joined to the ear canal opening. A wide band noise signal was introduced into the ear canal through one of the sound delivery tubes, and the output of the probe microphone used to calibrate the sound delivery system and the ER-10B microphone response *in situ*, to 50 kHz.

A set of measurements of normal emissions, taking 1–2 h, was then completed. For each single emission measurement, two tones (frequencies f_1 and f_2) were introduced into the ear canal through the two coupler tubes, and the ER-10B microphone output synchronously averaged, typically for 4 s. The lower-frequency stimulus amplitude, L_1, was always 10 dB higher than the higher-frequency stimulus amplitude, L_2 (Mills *et al.*, 1993; Whitehead *et al.*, 1995a, b). For this report, the basic measurement sequence involved a sequence of stimulus frequency ratios. The upper frequency, f_2, and both amplitudes of the two stimulus tones were fixed for these sequences, while the lower stimulus frequency, f_1, was incremented after each single measurement. For the initial data set for all animals, the basic sequence included measurements at 17 frequency ratios from $f_1/f_2=0.714$ to 0.925. There were additional measurements for some animals at some frequencies, particularly including lower ratios for $f_2=2$ kHz. The basic frequency ratio sequence was conducted for a number of different stimulus levels, starting near the noise floor for the cubic distortion tone (CDT, $2f_1-f_2$) emission. Stimulus levels were incremented in 10-dB steps, to a maximum of 80–100 dB SPL depending on age and f_2 frequency. This set of measurements was made for each of the following f_2 frequencies: 2, 8, 16, 32, and 48 kHz. Note that only for the older animals could emissions be detected above the noise floor for the higher frequencies (Mills and Rubel, 1996).

After the initial data set was completed, the animal was given an intraperitoneal (I.P.) furosemide injection to establish the ''passive'' emission response. Dosages were set on the basis of previous experiments (Mills *et al.*, 1993, 1994;

TABLE I. Number of animals in experimental groups. Age of each group is given in days after birth (dab) with the furosemide dosage used for that age group.

Age (dab)	Number	Furosemide (mg/kg)
15	6	60
17	6	80
20	6	100
25	5	150
30	4	200
42–46	3	300

Mills and Rubel, 1996) so that the cochlear amplifier would be rendered essentially nonfunctional for a brief time, usually about 5–7 min. The required dosages varied with age as noted in Table I. During the time that the cochlear amplifier was nonfunctional, a brief series of frequency ratio sequences was taken. Because of time constraints, these were limited to five or six different frequency ratios, and at stimulus amplitudes near the upper limits. The passive measurements were conducted for the same f_2 frequencies as the initial, active measurements, and 10-dB intervals were also used for the stimulus amplitudes. For the younger animals (up to 20 dab) the passive emissions were so weak that additional filtering was required to successfully detect them. A programmable low-pass filter was employed on the ER-10B output, to reduce the magnitude of the stimulus frequency components in the microphone signal, as was done previously (Mills and Rubel, 1996). This was successful in enabling detection of the amplitude of the weak passive signals but the phase response was not then usable, due to the additional phase shifts caused by the filtering.

Each animal was monitored for one half hour after injection to assure that there was at least partial recovery of emissions during this time. The total number of animals measured in each age group is given in Table I.

Procedures for the care and use of the animals reported on in this study were approved by the University of Washington Animal Care Committee (*re:* Grant No. NIH DC 00395, Ontogeny of Sensory Processes).

C. Data analysis: Signal delay

For this report, the variation in phase angle with stimulus frequency ratio (f_1/f_2) was generally converted into an equivalent ''signal delay.'' For an emission at the frequency given by $f_{mn}=(mf_1-nf_2)$, the round trip signal delays associated with that emission, T_{mn}, have been determined using the equation (Kimberley *et al.*, 1993; Mahoney and Kemp, 1995)

$$T_{mn}=-\Delta\Phi_{mn}/\Delta f_{mn}, \tag{1}$$

where the *corrected* emission phase angle, relative to the stimuli phases, is given by $\Phi_{mn}=\phi_{mn}-(m\phi_1-n\phi_2)$. The quantity ϕ_{mn} is the measured emission phase angle, and ϕ_1 and ϕ_2 the measured phase angles of the two stimuli at f_1 and f_2. All emission phase angles reported here are corrected emission phase angles.

The procedure employed has been to include a limited range of f_1 frequencies, typically 5–7 points, located around the amplitude maximum and spaced closely enough that the typical change in emission angle between neighboring ratios was securely less than 180°. The emission angles were then unwrapped by a computer program and the corrected, unwrapped phase angles were calculated and displayed. A linear, least-squares fit was then made to these points, and the round trip signal delay was calculated from the slope of this line, according to Eq. (1).

II. RESULTS

A. Signal delay, active emissions

We operationally define active emissions as those emissions which are emitted by a normal mammalian cochlea and which are essentially eliminated when the endocochlear potential is sharply reduced (Mills *et al.*, 1994; Norton *et al.*, 1991; Norton and Rubel, 1990). In practice, this means that these are certain odd-order emissions found at relatively low stimulus levels, although care must be employed in defining what stimulus levels are ''low'' for a given frequency, species, and developmental stage (Mills *et al.*, 1994). Representative results for amplitude and phase responses of active emissions at the cubic distortion tone (CDT, $2f_1-f_2$) frequency are shown in Fig. 1, for an adult gerbil. For each panel, the f_2 frequency and the stimulus levels L_1 and L_2 were kept fixed, and the f_1 frequency stepped in small increments. The amplitude of the emission is shown in the lower panel of each pair, and the phase angle response in the upper panel. Note that the horizontal axis in these figures is the ratio f_1/f_2. This is the most useful form for the axis for experiments in which f_2 is kept fixed, and f_1 varied. That is, a simple group delay leads to a linear relationship between phase and the f_1 frequency, or between phase and the ratio f_1/f_2.

The responses shown are typical of adult gerbils at relatively low stimulus levels. The amplitude responses had maxima at f_1/f_2 frequency ratios near 0.75–0.8, and the phase response was approximately linear across these middle f_1/f_2 ratios. There appeared to be a trend for the frequency ratio at maximum amplitude to move to lower ratios, for lower f_2 frequencies. This trend appeared stronger in younger animals, as the representative example of a 15 dab gerbil in Fig. 2 illustrates. Emissions are shown in Fig. 2 only for $f_2=2$ and 8 kHz, as adequate phase responses for stimuli with $f_2=16$ kHz and above were not obtainable in the 15 dab neonates, due to emission amplitudes being at or below the noise floor.

The range of frequency ratios chosen for the estimate of the signal delay in this experiment is also illustrated in Figs. 1 and 2, by the filled-in phase data points in the upper panels, and the horizontal bars in the lower panels. This range was chosen, on the basis of responses such as shown in Figs. 1 and 2, to be the best compromise across the range of f_2 frequencies from 2 to 32 kHz. This range, from $f_1/f_2=0.746$ to 0.82, includes the regions where the amplitude is maximum for nearly all ages, the exception being the younger animals for $f_2=2$ kHz. After the phase response was un-

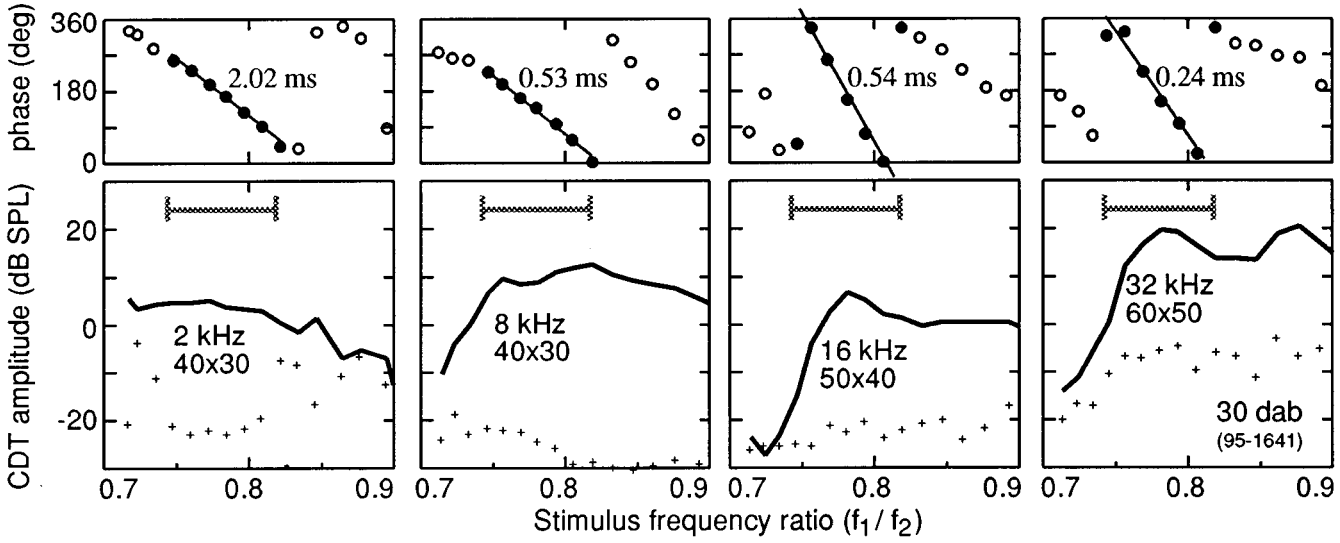

FIG. 1. Individual data: Amplitude and phase for the cubic distortion product (CDT, $2f_1-f_2$) emission are presented as a function of frequency ratio, for representative responses in a 30-day-old animal at low stimulus levels. The parameters listed in each of the lower panels are the upper stimulus frequency, f_2, and the stimulus levels, $L_1\times L_2$ (dB SPL). The crosses in the lower panels indicate the measured noise levels. The horizontal bars and the filled-in phase symbols indicate the phase responses that were used to calculate the round trip signal delay to be associated with that f_2 frequency and stimulus level pair. These are the seven measurements taken from $\phi_1/\phi_2=0.746$ to 0.820 in approximately 0.012 increments. The CDT phase angles listed here and elsewhere are those referenced to the measured stimulus phases, that is, by subtracting the quantity $(2\phi_1-\phi_2)$ from the measured CDT phase, where ϕ_1 is the measured phase angle of the $f1$ stimulus at the microphone, and ϕ_2 the phase angle for the f_2 stimulus. The phase zero reference in each panel is arbitrary. The least-squares best linear fit to the 7 phase points are shown by the lines, the figures give the derived signal delays in milliseconds (ms). Note that the phase angles are shown folded into a single 360° interval here, but were unwrapped before fitting the straight line shown.

wrapped, least-squares linear fits to the phase variation with frequency were made, as illustrated in Figs. 1 and 2. Values of signal delay from Eq. (1) corresponding to the slopes shown are also listed. These values are typical of the range found in these experiments, from 2 to 3 ms for $f_2=2$ kHz down to 0.2 ms for $f_2=32$ kHz. Note that, in contrast to the results for humans (Kimberley *et al.*, 1993), the phase angle response in gerbils was not always linear with frequency ratio, particularly outside the region of the maximum amplitude for the emission (Fig. 1).

The signal delays found varied not only with f_2 frequency, but with stimulus amplitude, $L_1\times L_2$, at a given f_2 frequency. Figure 3 presents the observed variation for the same two animals as in Figs. 1 and 2. Stimulus levels were incremented in 10-dB steps, with L_2 always 10 dB below L_1. As in these two examples, the derived signal delay typically decreased by a factor of 2 as the stimulus level increased

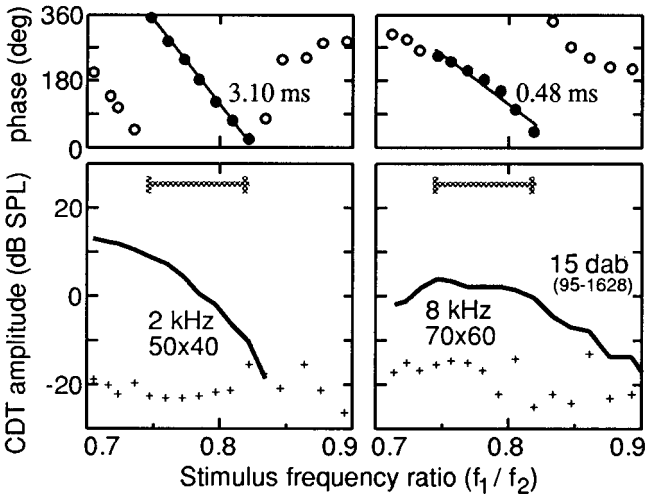

FIG. 2. Individual data: Amplitude and phase of CDT emissions are plotted versus frequency ratio, for representative responses in a 15-day-old animal at relatively low stimulus levels. Same conventions as Fig. 1. For these younger animals, adequate phase determinations for stimulus frequencies $f_2=16$ kHz and above were not obtainable.

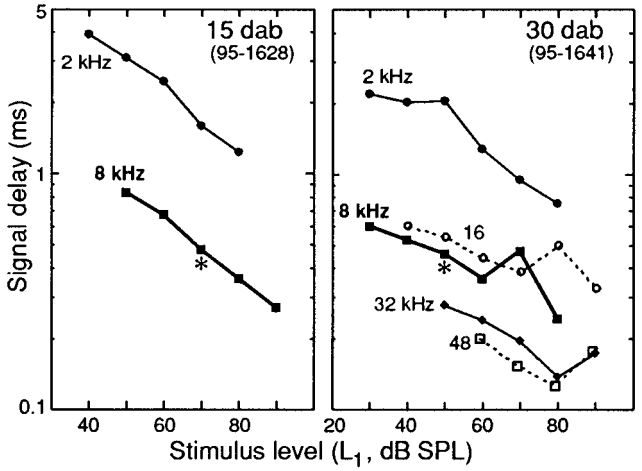

FIG. 3. Individual data: Round trip signal delay is presented as a function of stimulus level for the same individual animals presented in Figs. 1 and 2. The parameter listed is the higher stimulus frequency, f_2. Signal delays were calculated using the phase responses noted in Figs. 1 and 2. The asterisks denote ''equivalent'' stimulus levels for $f_2=8$ kHz (see text).

from 40 to 80 dB SPL. Further, there was no obvious tendency for the derived signal delay to reach an asymptote as lower or higher stimulus levels were approached. Finally, at higher stimulus levels were typically unexpected, systematic variations in the derived signal delays. For example, note the rapid variation in the derived signal delays for the 30 dab animal in Fig. 3, occurring between L_1=60 and 80 dB SPL for f_2=8 kHz, and between 70 and 90 dB SPL for f_2=16 kHz. These rapid minima and maxima were quite consistently found at higher stimulus levels, and the total variation in signal delay typically spanned about an octave.

These intrinsic variations with intensity make direct developmental comparisons very difficult. For example, the 8 kHz delay was typically larger for the 15 dab animal than it was for the 30 dab animal. This would seem to imply a *decrease* in signal delay at 8 kHz during development. However, it is known that higher stimulus levels are generally required in the ear canal at 15 dab compared to 30 dab to provide the same, equivalent levels in the cochlea at 8 kHz (Mills *et al.*, 1994; Mills and Rubel, 1994). Suppose this were a 20-dB difference, which is typical. The delay at 15 dab for, say, L_1=70 dB SPL should then be compared with the delay at 30 dab with L_1=50 dB SPL. These ''equivalent'' locations are marked with an asterisk in Fig. 3. It can be seen that, rather than showing a marked decrease with maturation, the comparison of such equivalent levels would imply that there was little change with maturation. There is a similar potential problem in comparisons between different f_2 frequencies at the same age, due to frequency variations in input transmission. For example, for the individual animal in Fig. 3, the 16-kHz delays were larger than the 8 kHz at the same levels (SPL). If middle ear effects accounted for a 20-dB difference at 16 kHz, however, one might correct the 16-kHz curve by shifting it to the left by 20 dB. The delays at 16 kHz at these ''equivalent'' levels would then be *smaller* than at 8 kHz.

In order to properly compare results, the stimulus levels employed for each animal were *normalized* by referencing them to the crossover stimulus level, L_X. This has been defined to be the stimulus level in the ear canal at which the transition from primarily active response to primarily passive response occurs in the cochlea (Mills *et al.*, 1994; Mills and Rubel, 1994). Values of L_X were estimated for each animal at each f_2 frequency as illustrated in Fig. 4.

The pre- and post-injection growth functions were obtained (with 10-dB stimulus intervals) for the three f_1/f_2 ratios noted, and were plotted as shown. The crossover stimulus level, L_x (and the shift, ΔC, used later) were calculated for each ratio as illustrated, and averaged over the three f_1/f_2 ratios.

The results in Fig. 3 can now be replotted in Fig. 5, with the stimulus level at each f_2 frequency now plotted relative to its crossover level, L_x, determined for that animal and f_2 frequency. The estimated ''threshold'' crossover levels for 2 kHz changed little between the 15 and 30 dab animals. This lack of improvement with age can be attributed to the open bulla condition of these experiments: In closed bulla conditions, the smaller bulla of younger animals typically causes an increase in the threshold measure, L_x, for frequencies

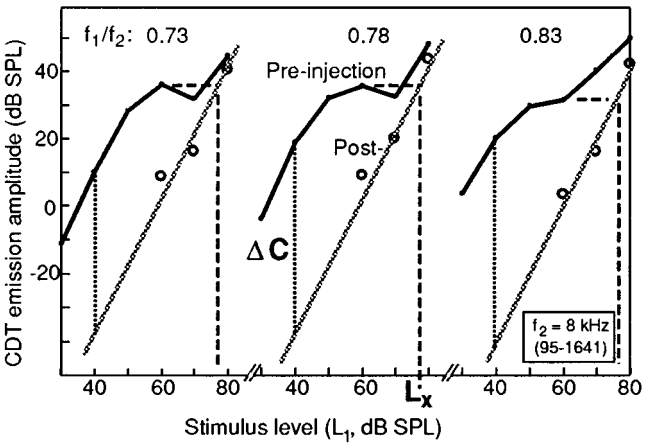

FIG. 4. Individual input–output, or ''growth functions,'' i.e., variation of cubic distortion tone (CDT, $2f_1-f_2$) emission amplitude with stimulus level. The solid lines are the normal, pre-injection growth functions. The post-injection responses, taken at the time of the ''flat minimum'' (Mills and Rubel, 1994), are indicated by the open circles, with the ''best-fit'' straight line with a slope of 2:1 shown. The three growth functions are all for f_2=8 kHz, but different ratios of stimulus frequencies, f_1 and f_2, as noted. Each of these growth functions yields an estimate of the active–passive transition level, L_x, and the shift in emission amplitude at low signal levels, labeled ΔC. The shift, ΔC, is the difference between the CDT emissions with and without a functional cochlear amplifier, and is therefore related to the gain of the cochlear amplifier. For the example shown, L_x was 77 dB SPL for all three ratios, and the average shift, ΔC, was 55 dB.

below 4 kHz (Mills *et al.*, 1994). At 8 kHz, there was a decrease of nearly 20 dB for the older animal compared to the 15 dab animal.

The relative maxima and minima seen in the 30 dab animal in Fig. 3, which did not occur at the same absolute levels, are now observed in Fig. 5 at approximately the same levels *relative* to the crossover stimulus level. These rapid variations occurred largely in the 10-dB range for which the stimulus levels L_1 were 10 dB below the crossover level, L_x,

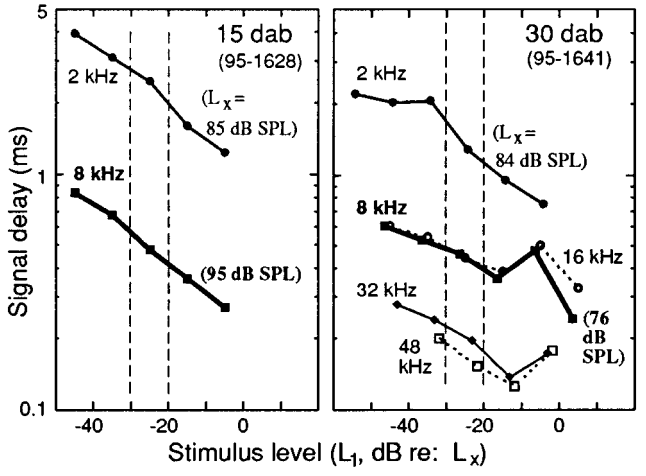

FIG. 5. Individual data: Round trip signal delay is plotted as a function of *relative* stimulus level. This is the same data as in Fig. 3, but now the stimulus levels are plotted relative to the active–passive transition levels, L_x. These were determined for each animal at each f_2 frequency using growth function plots as illustrated in Fig. 4. The estimated values of L_x are shown, in parentheses, for f_2=2 and 8 kHz. The vertical dashed lines simply note the relative levels 20 and 30 dB below L_x.

and equal to it (i.e., between $L_1=L_x-10$ dB and $L_1=L_x$). It seems likely, therefore, that the rapid variation observed was associated with the transition between ''active'' and ''passive'' responses. That is, the rapid variation could be attributed to a phase interaction between these two components. This interpretation was further supported by the observation that, among individual animals of the same age, the rapid variation always occurred near the transition level L_x, but the form varied, sometimes dominated by a very sharp minimum, and at other times there was a more moderate maximum (i.e., like that shown in Figs. 3 and 5).

It seems obvious that, to study the developmental variation of the signal delay in the normal, *active* cochlea, this active–passive transition region should be avoided. For this report, we have therefore chosen to report results in signal delay primarily for the stimulus level which is 30 dB below L_x. This level is far enough from the active–passive transition that it seems little affected by it, yet the stimulus level is typically still high enough to yield adequate signal to noise ratios. The level 20 dB below L_x would work as well; we have made all the calculations presented below for this case as well, and the results are virtually identical.

In Fig. 6(A) and (B) we present the variation with age for the mean signal delay, measured at the stimulus level estimated to be ''constant'' in the cochlea. That is, for each individual animal the signal delay was estimated at a stimulus level 30 dB below the crossover level, L_x, as in Fig. 6. The estimated delays were then averaged across animals within an age group. For comparison, in Fig. 6(C) we present the mean delays measured at a constant stimulus level as measured in the ear canal, in this case for $L_1\times L_2=50\times 40$ dB SPL.

The overall trend is obvious: there was a modest decrease in signal delay between 15 and 20 dab, at all f_2 frequencies. The decrease was not as large, however, when comparison was made with stimulus levels at a constant offset to characteristic levels in the cochlea [Fig. 6(A) and (B)] compared to delays measured at the same absolute levels in the ear canal [Fig. 6(C)]. The reason is that the *relative* stimulus levels corresponded to higher ear canal stimulus levels in the younger animals than the older (except at 2 kHz where there was little change with age). Note that there were no measurable emissions available for stimulus levels of 50 dB SPL for younger animals at higher frequencies, because of the increase in passive threshold (Mills *et al.*, 1994).

Note also in Fig. 6(A) that there was an interesting minimum in the derived signal delay that occurred early in development for most f_2 frequencies. This minimum was observed first at 2 kHz, at about 17 dab, and then at 8 kHz at about 20 dab, and finally at 32 kHz at about 25 dab. In all cases, the minimum occurred several days to a week after the emissions first become measurable at the corresponding frequency.

The dashed line in Fig. 6(A) displays the difference between the mean signal delay for stimulus frequency $f_2=2$ kHz and that for $f_2=8$ kHz. This difference is obviously dominated by the variations in the signal delay for $f_2=2$ kHz.

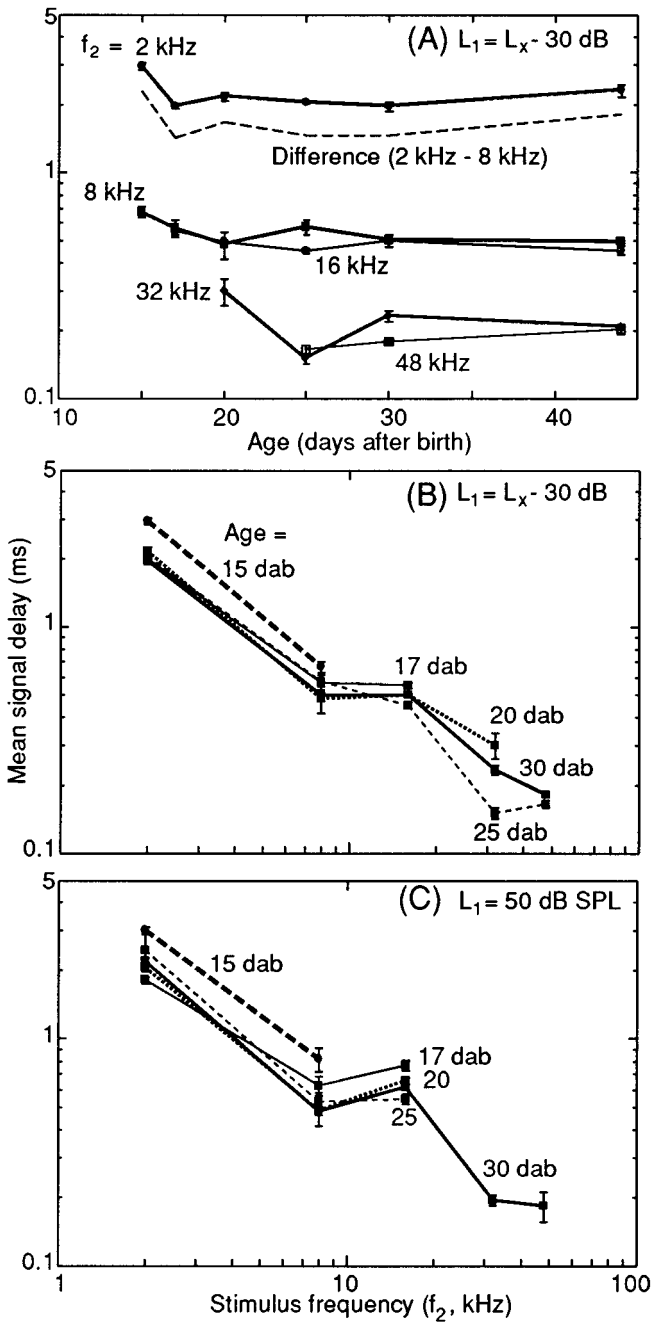

FIG. 6. Group data, giving the variation with age of the mean signal delay. Bars indicate standard error of the mean. (A) Delay estimated for each individual at the stimulus level L_1 which was 30 dB below the active–passive level, L_x, for that individual, and then averaged. The parameter is the stimulus frequency, f_2. The dashed line shows the *difference* in the mean delay between that for $f_2=2$ kHz and that for $f_2=8$ kHz. (B) Same data as (A), plotted to show the variation with f_2 frequency, with age as the parameter in days after birth (dab). (C) Mean delay measured at the stimulus level $L_1=50$ dB SPL, for all frequencies and ages. Note that emission amplitudes for higher frequencies at this stimulus level were not above the noise floor in the younger animals.

B. Variation of amplitude with f_1/f_2: The ''filter'' response for active emissions

Measurements were made of the normal, pre-injection emission amplitudes as a function of stimulus frequency ratio for fixed frequency f_2 of 2, 8, 16, and 32 kHz. Typical

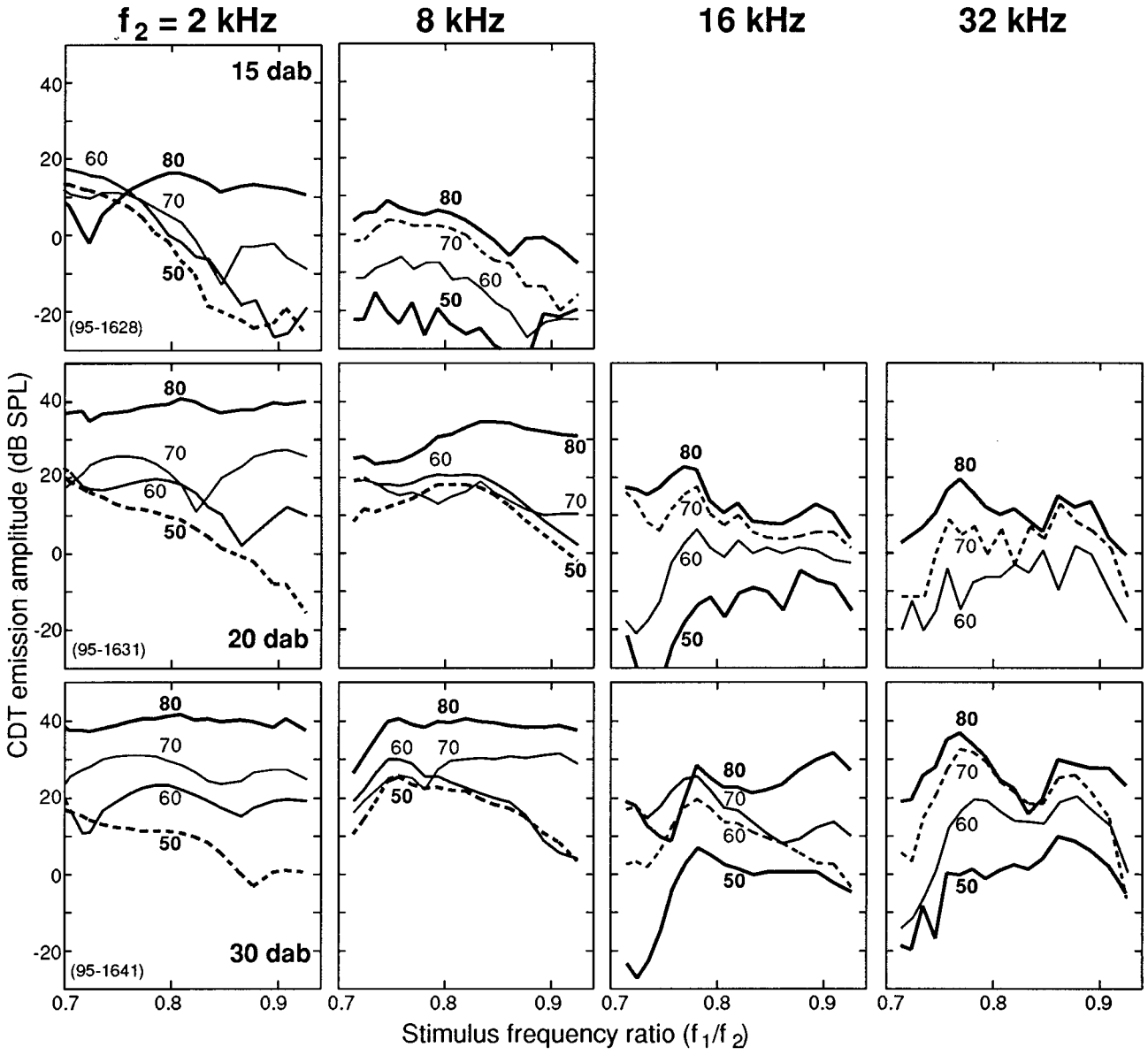

FIG. 7. Variation of cubic distortion tone (CDT, $2f_1-f_2$) emission as a function of stimulus frequency ratio (f_1/f_2), for individual animals at three different ages. The parameter listed is the stimulus level, L_1, in dB SPL. For all data, the higher-frequency stimulus level, L_2, was 10 dB below L_1. For clarity, only results for stimulus levels for L_1=50 to L_1=80 dB SPL are shown, and the extremes are shown in heavier lines. The line in each panel which is dashed indicates the emission for the stimulus level which falls between 20 and 30 dB below the active–passive transition level, L_x, illustrated in Fig. 4.

results are presented for the CDT emission for three individual animals in Fig. 7. For clarity, only the emission distributions for stimulus levels from L_1=50 to 80 dB SPL are shown.

Over the frequency ratio covered, there were often two main peaks in the emission amplitude. There was no obvious, consistent trend for the emission amplitude to ''flatten'' with increasing stimulus level, i.e., the amplitude did not tend to become constant with frequency ratio at high stimulus levels. There also was no consistent change with stimulus level for the frequency ratios associated with the amplitude maxima, i.e., the ''peak center frequency.''

This ''filter'' behavior is examined more closely in Fig. 8, where the mean CDT ($2f_1-f_2$) and fifth order amplitudes ($3f_1-2f_2$) are presented. For this plot, the horizontal axis employs the *emission frequencies* (*re*: f_2) rather than the stimulus frequency ratio. This is done to better compare these two odd-order terms, because it is well known that at least some of the peaks in the odd-order emission amplitudes coincide when plotted against emission frequency, rather than against stimulus frequency (Brown and Gaskill, 1990b; Fahey and Allen, 1986; Gaskill and Brown, 1990). To obtain the mean filter responses, individual filter functions were first defined by normalizing the emission components of each individual animal to the maximum values occurring in the interval observed. The normalized values were then averaged over the age group, and the mean results presented in Fig. 8. For comparison between different ages, at each age we present only the emission distribution for one stimulus level pair, choosing in each case the stimulus level falling between

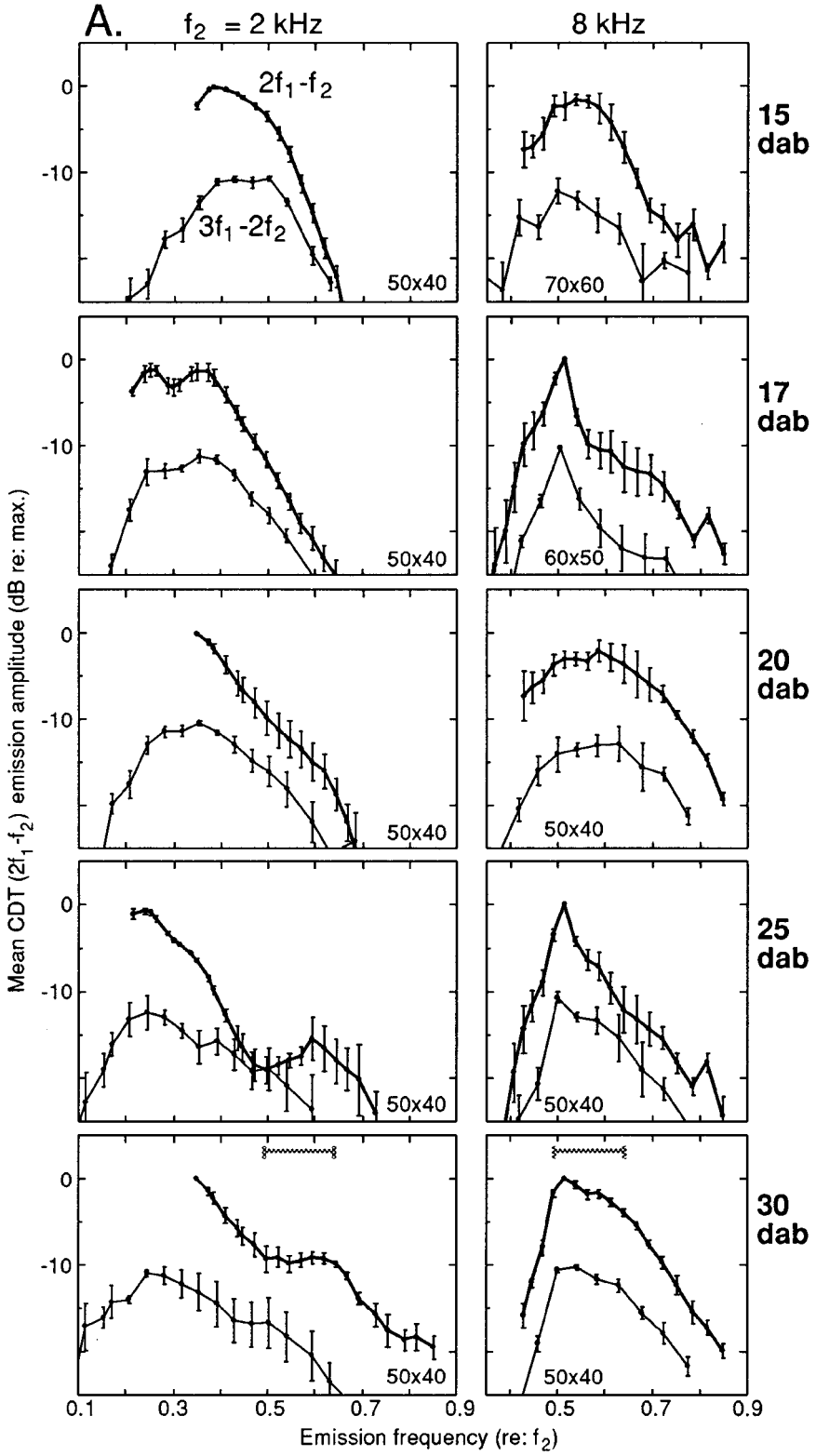

FIG. 8. A, B. Group data. Mean ''filter'' responses for emissions at $2f_1-f_2$ and $3f_1-2f_2$: Variation of emission amplitude when stimulus frequency ratio is changed, for the stimulus levels ($L_1\times L_2$, dB SPL) listed in the lower part of each panel. Stimulus levels L_1 were chosen which were between 20 and 30 dB below the mean active–passive level, L_x, for that age group. (See Fig. 5.) In each panel, the vertical axis is the response (in dB) relative to the maximum emission. That is, individual amplitude responses were normalized to the maximum response for each animal over the measured frequency interval at a given f_2, before averaging over the age group. The 0-dB reference levels are indicated: note the emission for $3f_1-2f_2$ is displaced 10 dB below $2f_1-f_2$ for clarity. The horizontal axis is the *emission* frequency relative to the f_2 frequency, not the stimulus frequency ratio as in Fig. 7. For the $2f_1-f_2$ emission, the horizontal axis is chosen to be equivalent to that for Fig. 7; note, however, that *different* values of f_1/f_2 are associated with the responses for $3f_1-2f_2$ compared to $2f_1-f_2$ at the same position on the horizontal axis. Vertical bars represent standard errors of the mean. For reference, the horizontal bars in the row for the 30 day after birth (dab) animals indicate the frequency range which was employed to determine the signal delays (for all ages) from the phase change of the $2f_1-f_2$ emission, at approximately the same stimulus levels, in the previous section.

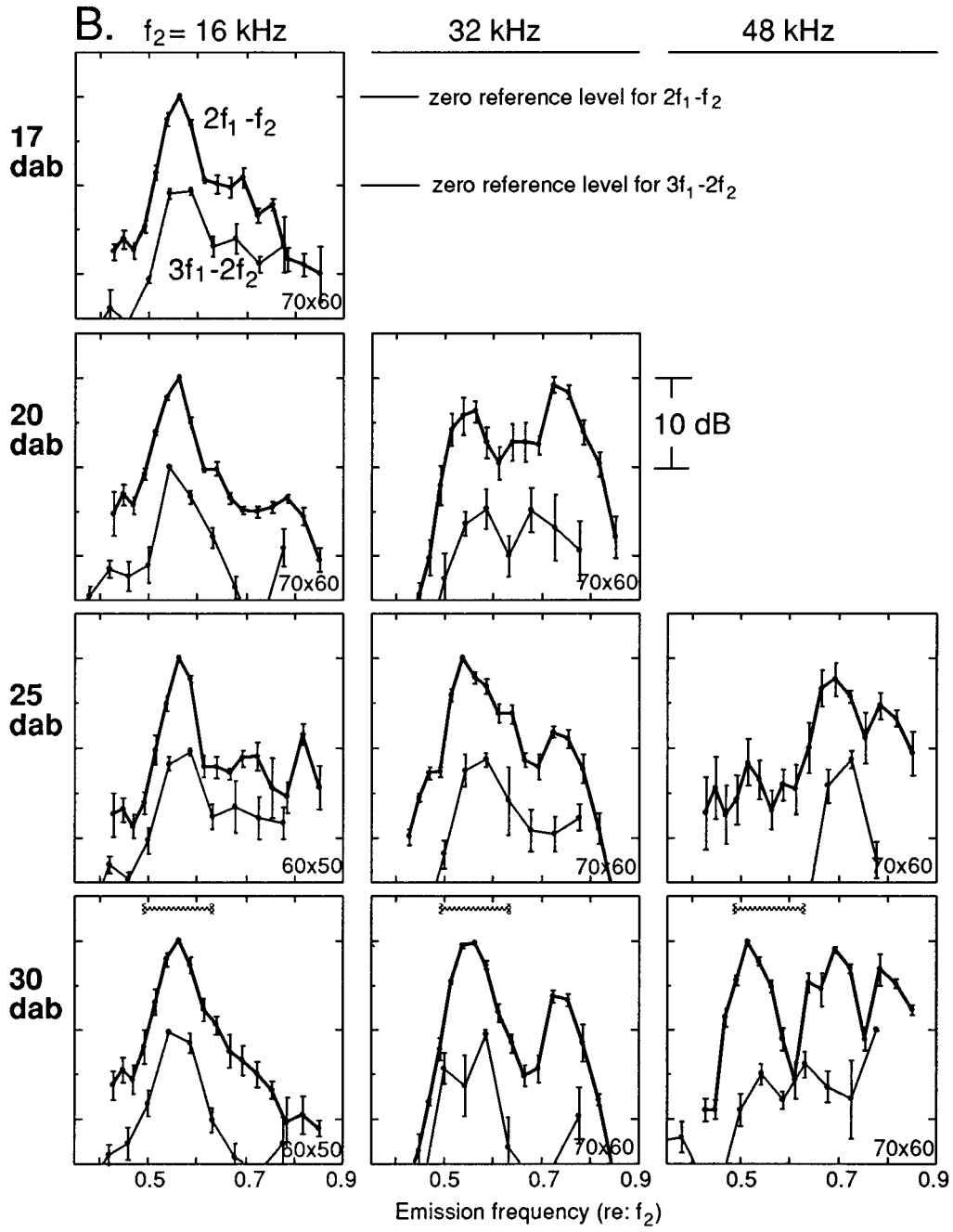

FIG. 8. (*Continued.*)

the mean values for $(L_x-30$ dB) and $(L_x-20$ dB) for the age group. That is, we display in Fig. 8 approximately the same stimulus levels that were used in estimates of signal delays in the previous section, so that these amplitude results can similarly be considered characteristic of active processes. The stimulus level pairs chosen are listed at the bottom of each panel. The horizontal bar in the lowest panels indicates for reference the frequency ratios which were employed to obtain the signal delay information from the CDT emission in the first section, for all age groups. The upper, heavier line in each panel denotes the $2f_1-f_2$ component. The lighter line is the $3f_1-2f_2$ component, with its zero reference shifted 10 dB downward for clarity.

The ''second filter'' effect is dramatically displayed in this presentation. The higher order term, $3f_1-2f_2$, had peaks at exactly the same *emission* frequencies as did the cubic term, $2f_1-f_2$. Further, at the stimulus levels chosen, there was generally only one primary peak in the *mean* emission for the adult animals. This primary peak location and shape was typically very consistent from animal to animal in a given age group. This is evidenced by the very small variances typically found around the peak values. Note that if the normalized mean amplitude at the primary peak was 0 dB, i.e., exactly equal to the reference level with zero variance, it means that the maximum amplitude occurred at the same emission frequency for *every* animal in that group. Many of the mean primary peak values were equal to or very close to

0 dB, illustrating the remarkable consistency of the frequency of maximum emission within each age group. The ''center frequency'' of the primary peak (frequency of emission maximum) was close to $0.5f_2$ for all f_2 frequencies, except for f_2=2 kHz.

Developmentally, there was no significant change in the mean center frequencies for these emission peaks across age groups from 15 dab to 30 dab. There was also no obvious trend for the peaks to become sharper as the animal matured.

In addition to these peaks, there were other peaks observed at most ages. These other peaks were most obvious for stimulus frequencies above 2 kHz, where they were typically found with emission frequencies of $0.6f_2$ to $0.7f_2$. These peaks were often relatively stronger at younger ages, frequently strong enough to be the dominant peak.

The mean results for f_2=8 kHz were more complex. There was an apparent broad maximum at 15 dab, which became quite sharp at 17 dab, but then broadened again at 20 dab, only to change again into a sharp peak at 25 dab. The emission frequency at the maximum amplitude also appeared to change with age in a complex manner. Figure 9 presents the individual observations which made up these mean distributions, for more detailed analysis.

The individual distributions show that the broad peaks in the mean data could generally be considered to consist of the combination of two close, partly ''resolved'' peaks, rather than a single broad peak. These typically consisted of a single peak at an emission frequency of $0.5f_2$, and another peak at about $0.6f_2$. At most ages, the first peak was the dominant one. At 20 dab, however, the first peak appeared to be relatively and absolutely weaker than at other ages, to the extent that it was not detectable in comparison to the second peak in three of the six animals in this age group. At 25 dab, all of the animals had very similar amplitude distributions, having a single sharp maximum at about $0.5f_2$. There were at least some animals in every age group with a peak of similar sharpness occurring at nearly the same frequency ($0.5f_2$). The exception was the youngest age: At 15 dab, no single animal had a peak that sharp, although most animals did have a peak with a relative maximum at an emission frequency near $0.5f_2$.

The overall behavior of the peak responses are summarized in schematic form in Fig. 10. Here, we include the mean peak frequencies, relative amplitudes, and peak widths for all animals in this study. Each panel represents one stimulus frequency, f_2, in which the age of the animal increases downward. The frequencies at maximum amplitude have been determined for individual responses and then averaged over each age group. The ''center'' frequency variances were quite small, typically about $0.01f_2$. The relative mean peak amplitudes, essentially the same as in Fig. 8, are indicated by the size of the symbol at the peak frequency (see key). The horizontal bars represent the widths of the peak, measured 10 dB below the peak maximum. Because of the interference in the response caused by neighboring peaks (e.g., Fig. 9) the two ''half-widths,'' i.e., the distances from the emission frequency at maximum amplitude to the frequencies where the emission was 10 dB lower, were determined for each individual peak. The *smaller* half-width was

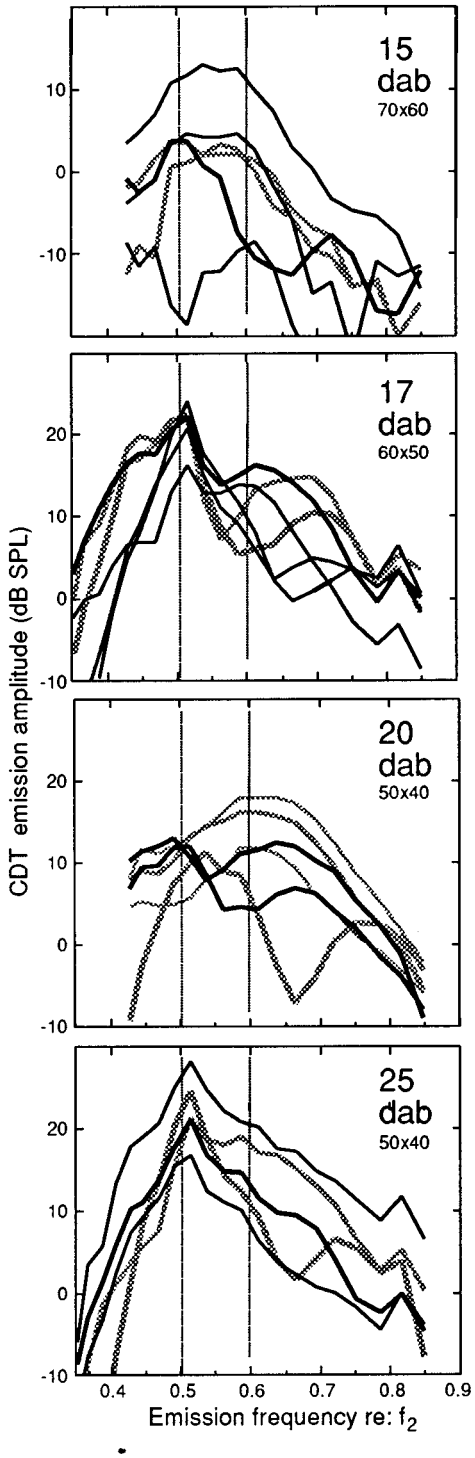

FIG. 9. Individual responses for all animals in each age group for f_2=8 kHz. The stimulus levels chosen were the same as Fig. 8 (i.e., L_1 was between 20 and 30 dB below L_x) and are indicated in each panel ($L_1 \times L_2$, dB SPL). For clarity, some individual responses are emphasized by different line intensities. The two vertical lines, at $0.5f_2$ and $0.6f_2$, are for reference.

used to obtain the means shown. The stimulus levels were the same as in Fig. 8, that is, between 20 and 30 dB below the crossover level, L_x.

There was a consistent developmental trend for f_2=2 kHz. There was one prominent peak at relatively low emission frequencies, which shifted to even lower emission frequencies as the animals matured. The peak width, however,

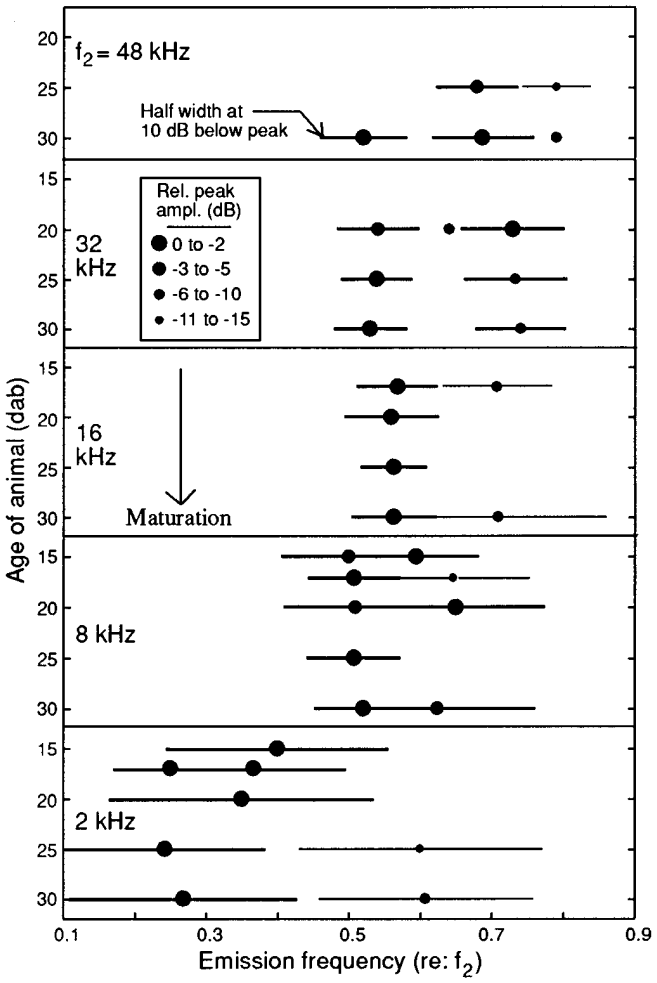

FIG. 10. A schematic illustrating the development of mean peak amplitudes, center frequencies, and half widths. Center frequencies of each peak were determined for the individual responses, and averaged to obtain the mean peak frequencies shown. The relative mean amplitude of each peak was determined from Fig. 8, and is denoted by the size of the symbol, according to the key. The horizontal bars are not the variances of the center frequencies, which were much smaller. Rather, the bars represent the width of the amplitude response at the point 10 dB down from the maximum. Strictly, since the peaks were usually asymmetrical, often because one side was obscured by the presence of a neighboring peak, the *smaller* of the two estimated ''half'' widths of each individual peak was used to obtain the mean half widths. Widths are not shown for two of the peaks, because the response was too confused by noise or by stronger neighboring peaks.

changed little during this time. There was also a secondary peak at about $0.60f_2$ which appeared as the animals matured. It should be noted that this peak could have been ''present'' earlier, but obscured by the much stronger, lower-frequency peak.

For higher frequencies, there was no general trend noted in the variation of peak frequencies or widths with development. For f_2=8 kHz, for example, there was a prominent peak near $0.50f_2$ at all ages. Neither the center frequency nor the peak width changed consistently with age. Similar results hold for f_2=16 kHz, which had a very sharp, stable peak at $0.56f_2$ at all ages, and for f_2=32 kHz, which consistently had two peaks near $0.50f_2$ and $0.70f_2$. The observed peaks could be quite sharp, with half widths at the 10-dB level as small as $0.04f_2$.

C. Relationship of active and passive emissions

Passive emissions are operationally defined as those emissions measured at the time of maximum furosemide effect, when the endocochlear potential has been substantially reduced (Mills *et al.*, 1994; Mills and Rubel, 1994). These passive emissions are almost always weaker than (or equal to) the normal emissions (e.g., see Fig. 4) and can only be measured during a brief time. The measurement of passive emissions is therefore more difficult than for the active emissions. In particular, in this experiment, for the younger animals the passive emissions were generally so weak that the programmable filter had to be employed, to reduce the amplitude of the much stronger primaries prior to A/D conversion (see Sec. I). This resulted in the loss of accurate phase information for the passive emissions from the younger animals, so that the passive signal delays could not generally be calculated. Further, even with the additional filtering often only the passive emissions near the maximum amplitude (as a function of stimulus ratio) could be adequately measured. While there was enough information available on all animals to estimate cochlear amplifier gains and active–passive stimulus levels (as in Fig. 4), there was only sufficient information to adequately characterize the passive emission amplitude distribution and the signal delay for several individual, mostly older animals.

The question naturally arose, in seeing the sharp peaks in the active emissions (Fig. 8), whether the same peaks occurred in the passive, post-injection response at similar stimulus levels. This might already be concluded, since we have already shown: (1) that the emissions at high stimulus levels are typically little changed by furosemide intoxication (Fig. 4), i.e., the pre-injection and passive emissions have the same distribution at high stimulus levels; and (2) there is little change in the pre-injection emission amplitude distribution comparing high and low stimulus levels (Fig. 7). One might also like to compare the two distributions directly, by comparing the pre-injection emissions at low stimulus levels with the ''passive,'' post-injection emissions at the same stimulus levels, i.e., 20–30 dB below L_x. This is not strictly possible, because the passive emissions are simply not measurable at these stimulus levels (e.g., Fig. 4). As a compromise, in Fig. 11 we have compared the active, pre-injection emissions at low stimulus levels for several individual animals to the post-injection, passive emissions measured in each individual *at a stimulus level 30 dB higher*.

Using the established slope of 2:1 for the passive emissions, the passive amplitude distribution was then shifted downward by 60 dB, so that it would represent the amplitude expected at the *same* stimulus level as the active emissions (Mills *et al.*, 1994; Mills and Rubel, 1996). The amplitude scales of the active and passive emissions are given on the left and right sides of Fig. 11, respectively. The scales have been set so that the active and passive emissions would overlap in these panels *if* the difference between the active and passive emission amplitudes at the same stimulus level was 40 dB. For the stimulus levels we are considering here, this would generally occur if the shift ΔC was 40 dB (Fig. 4).

It is clear from Fig. 11 that the major peaks observed in the active emissions (as a function of stimulus frequency)

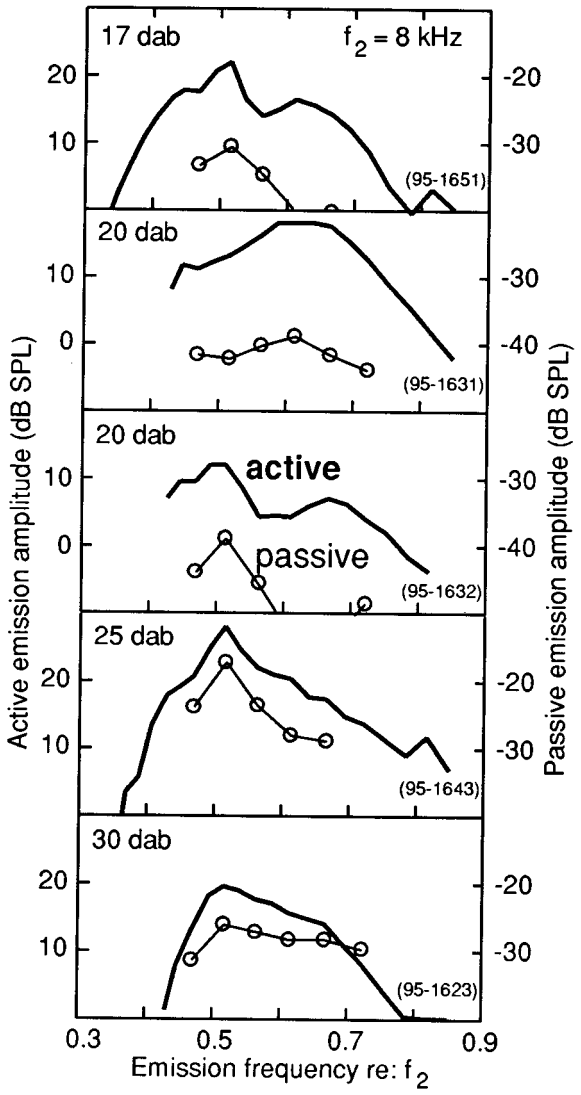

FIG. 11. Comparison of active and passive ''filter'' responses for individual animals at f_2=8 kHz. For the active responses at each age, the stimulus levels were the same as that in Figs. 8 and 11, i.e., were $L_1 \times L_2$=60×50 dB SPL for the 17 dab animal, and 50×40 dB SPL for the others. The passive responses were obtained at the time of maximum furosemide effect, at the stimulus level 30 dB higher than that for the active response. The passive responses were then extrapolated down to the same stimulus level as the active, using the established 2:1 slope, i.e., 60 dB was subtracted from the observed amplitude. The scales for the active and passive amplitudes are different, and are on the left and right sides, respectively. The scales were set so that, if the data points overlapped, the active emission amplitude was 40 dB more than the passive amplitude (i.e., if the active and passive points overlapped, A_c=40 dB).

were typically found in the passive, post-injection emissions as well. There was sometimes a modest difference between active and passive emission distributions in the *relative* peak amplitudes noted in individual animals. For example, in the 17 dab animal, the peak at higher frequencies (at 0.62 f_2) was relatively larger for the active emission than for the passive. This is probably related to the similar shift in the relative amplitudes of the peaks of the normal emission as a function of stimulus level (Fig. 7).

Data such as that in Fig. 11 lead to the following conclusion: For low stimulus levels, when the cochlear amplifier

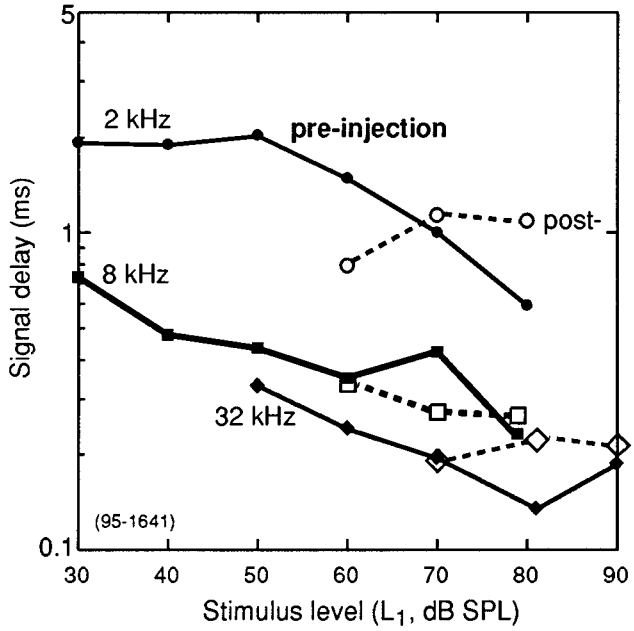

FIG. 12. Comparison of signal delay pre- and post-injection for an individual 30 dab animal. The parameter listed is the f_2 frequency. The pre-injection data are shown in filled symbols and solid lines, the post-injection in open symbols and light lines. Note that the symbols for f_2 frequencies of 8 and 32 kHz at a stimulus level of 80 dB SPL are displaced slightly in the horizontal direction so that they do not overlap.

was made inoperative by furosemide, the entire emission amplitude pattern shifted downward. The amount of shift in amplitude was approximately the same at all stimulus frequency ratios, and was equal to the shift, ΔC, at that f_2 frequency.

Finally, Fig. 12 displays the signal delay found as a function of frequency for a typical young adult animal. The pre-injection results are shown in solid lines and filled-in symbols, with the post-injection delays shown in shaded lines and open symbols.

As noted earlier, the pre-injection signal delays generally decreased as the amplitude increased. That is, the delays associated with the active process (the normal emissions at low stimulus levels) were larger than those found at higher stimulus levels. The signal delays for the pre-injection high level emissions, on the other hand, were very similar to the passive emissions as defined here. The signal delays for the post-injection, passive emissions, on the other hand, did not appear to change consistently with a change in stimulus level, at least over the relatively limited range over which the passive emissions could actually be measured.

III. SUMMARY OF RESULTS

The response of the cochlea as a function of stimulus frequency ratio (f_1/f_2) is complex; it is complex at a given developmental age and the response changes with age in a complex, often counterintuitive manner. The complexity applies both to changes in phase angle, including the derived signal delay for a narrow range of stimulus frequency ratios, and to changes in amplitude, including the observed multiple peak structure. Because of this complexity, it seems useful

here to summarize the data obtained in this study. The goal here is to provide a *tentative* summary of the most salient features of the results to date. Obviously, many of these trends require confirmation.

(1) The active and passive emissions have essentially the same signal delay and amplitude response as a function of f_1/f_2 (Figs. 11 and 12). That is, the normal signal delay measured at high stimulus levels is about the same as that measured at the same high levels when the cochlear amplifier is temporarily nonfunctional. Further, the passive amplitude/filter response is similar in form to the active filter response at *all* stimulus levels. In other words, when furosemide is injected, the emission amplitudes for low stimulus levels shift downward by approximately the same amount, relatively independent of stimulus frequency ratio. However, the signal delay associated with the active response (i.e., the normal delay at low stimulus levels) is higher than that associated with the passive or active responses (at high stimulus levels). The passive signal delay at low stimulus levels is not measurable.

(2) At a given age, the signal delay of active emissions typically decreases with increasing stimulus level, except for a complex response at high stimulus levels (Figs. 3 and 12).

(3) This complex response is associated with the active–passive transition (Figs. 4 and 5). It seems useful to compare responses at different frequencies and ages not on the basis of the absolute stimulus levels, but on the basis of the stimulus levels *relative* to the active–passive transition level, L_x. The comparison seems valid for both phase and amplitude responses. To study active emission characteristics, responses for stimuli well below the active–passive transition should be compared.

(4) At all frequencies, the derived signal delay typically *decreases* only slightly during maturation. However, there is an interesting relative minimum in signal delay which occurs, first at lower frequencies and subsequently at higher frequencies [Fig. 6(A)].

(5) The changes in signal delay with development are relatively small and complex. Depending on the observed frequencies and age groups, increases or decreases could be measured [Fig. 6(B)]. In terms of its signal delay characteristics, the cochlea in gerbils seems essentially mature near hearing onset.

(6) The difference in signal delay between low and high frequencies appears to be dominated by intrinsic changes in the larger, low-frequency delay [Fig. 6(A)].

(7) The structure in the amplitude responses, as a function of stimulus frequency, changes little with stimulus level at any age (Fig. 7). This includes the peak center frequencies, which are essentially unchanged with stimulus level. The peaks can be quite sharp, with mean half widths at 10 dB below the peak as small as $0.04f_2$.

(8) At all ages, the responses of the CDT at $2f_1-f_2$ and the fifth order term at $3f_1-2f_2$ are very similar when plotted as a function of the *emission* frequency (Fig. 8). The most prominent peak in adult animals is usually at an emission frequency between $0.5f_2$ and $0.6f_2$.

(9) For $f_2=2$ kHz, the center frequency of the most prominent peak decreases with age. At higher frequencies, the center frequencies of individual peaks are quite stable, but there may be a modest tendency for the relative amplitudes of the peaks to change with age. That is, the most prominent peaks in the younger animals tend to be those at higher frequencies (closer f_1 and f_2 frequencies), while those in adult animals tend to be those at the mid-frequencies (emission frequencies equal to $0.50f_2$ to $0.60f_2$). There are no consistent developmental trends in the peak center frequencies or peak widths themselves (Fig. 10).

(10) Overall, except for the changes noted at 2 kHz, the cochlea appears essentially mature near hearing onset in the characteristics which account for the variation of amplitude and phase with stimulus frequency ratio.

IV. DISCUSSION

A. Decrease in signal delay with stimulus intensity

From simple arguments, the largest component of the observed signal delay for distortion product emissions in the mammalian cochlea is likely to be phase buildup in the ingoing traveling wave associated with the stimulus (Eggermont, 1979; Mahoney and Kemp, 1995). Our observation that the relative signal delays change very little with development (Fig. 6) implies that the cochlear amplifier is mature in this respect even at early ages. The phase changes due to place code shifts are presumed to be comparatively small, and so not detected in these measurements.

It is well known that the signal delay, calculated by Eq. (1) from the observed phase changes, decreases with increasing stimulus intensity (Brown and Kemp, 1985). The observed behavior apparently follows from two simple assumptions:

(1) The cochlear amplifier reaches nonlinear levels even for generally small stimulus levels. For typical mammalian ears, the saturation of the cochlear amplifier begins to produce signal compression at such low signal levels that the existence of a linear regime cannot easily be established (Brown, 1993; Goldstein, 1967).

(2) The dominant odd-order emissions are generated in areas where the cochlear amplifier operation has become nonlinear. This assumption rests on the idea that the nonlinear mechanism which produces the emission is intrinsically related to, or is the same as, the nonlinear mechanism which causes the saturation. This idea has support in measurements which show that the vulnerable odd-order emissions are intrinsically tied to the mechanisms which produce amplification and sharp tuning at low stimulus levels (Rübsamen *et al.*, 1995). This assumption is further based on the simple idea that the dominant distortion will be produced where the response amplitude is sufficient to involve nonlinear mechanics.

The traveling wave phase buildup is very rapid as the peak of emission is reached (Robles *et al.*, 1986; Zweig, 1991). As the stimulus level increases, the cochlear amplifier saturation begins to occur more and more basally (Johnstone *et al.*, 1986). If the centroid of the emission generation area moves even slightly basally with increasing stimulus levels, this effect could easily cause a reduction in the apparent signal delay with increasing stimulus intensity.

B. Interpretation of development of signal delay characteristics

For these measurements, the frequency f_2 was fixed and the frequency f_1 varied. The argument for this approach is that the location of the site of emission generation in the cochlea, at least for weak stimuli, is believed to be near the f_2 place (Brown and Kemp, 1984; Kummer *et al.*, 1995; Martin *et al.*, 1987). That is, the emission originates near the location on the BM where a single weak tone of frequency f_2 would have a maximum response. There is then some hope that varying f_1 while fixing f_2 would leave the physical site of generation approximately unchanged, allowing for a simpler interpretation of the phase changes (Mahoney and Kemp, 1995).

Even for constant stimulus levels and f_2 frequency, the derived signal delay depends somewhat on the frequency ratio. For practical and theoretical reasons, it has been usual to determine the signal delay for a small range of stimulus frequency ratios which span the ratio where the emission amplitude is a maximum. For this report, we chose a single range in stimulus frequency ratio which was a compromise, approximately spanning the CDT emission maximum as a function of f_2. Other possibilities exist. We could, for example, have changed the frequency ratio for each f_2, and even for each age. This seems like a needless complication, which could potentially confuse the interpretation. In any case, the center frequencies of the peaks turned out to be insensitive to age.

Using either the phase delay at constant stimulus levels in the ear canal [Fig. 6(C)] or at a constant level below the active–passive transition level [Fig. 7(A) and (B)], we found a modest decrease in signal delay with age. Such a decrease, found in human infants, was used to suggest that there was no shift of the place code after this age (Brown *et al.*, 1994). However, this conclusion does not appear to be justified by these results, for the following reasons. In gerbil, the shift in the place code for mid- and high frequencies appears to be primarily due to a shift in the passive cochlear response, associated with a shift in the passive base cutoff frequency (Mills *et al.*, 1994). The place code shift merely moves the place on the BM where the cochlear amplifier begins to respond at a given frequency. The phase buildup associated with the passive, linear response of the incoming stimulus, *up to the point where the cochlear amplifier starts to respond*, is estimated to be relatively small compared to the phase buildup associated with the cochlear amplifier (Eggermont, 1979; Zweig, 1991). Therefore, it is expected that developmental changes in the cochlear amplifier itself would dominate signal delay changes, masking any effects due to place code shifts.

This expectation appears to be supported by the data found for the gerbil. After normalizing the stimulus levels to the active–passive transition, there was very little change in signal delay found with age. During the same period, it is known that there are significant changes in the place code in the base of the gerbil cochlea (Arjmand *et al.*, 1988; Harris and Dallos, 1984; Mills *et al.*, 1994; Mills and Rubel, 1996). Unless there were fortuitous, and unlikely, compensating changes, the known place code changes cannot have affected the signal delay very much. We conclude that, in gerbils at least, the place code shift cannot be determined by measurement of the signal delay characteristics. In addition, we conclude that from the onset of hearing to adulthood in gerbils, there are relatively small changes in the cochlear properties which are responsible for the signal delay characteristics. In contrast, there are other characteristics of the cochlea and cochlear amplifier which change considerably during the same period (Sanes *et al.*, 1989; Rubel, 1978; Rubel and Ryals, 1983; Walsh and Romand, 1992; Woolf and Ryan, 1984; Woolf *et al.*, 1986; Yancey and Dallos, 1985).

C. Emission amplitude response as a function of stimulus frequency ratio

We have described an amplitude response in gerbils with multiple maxima at emission frequencies of $0.50f_2$ to $0.75f_2$, at f_2 frequencies of 8 kHz and higher (Figs. 8 and 12). The relative amplitudes of the peaks seem to change with stimulus level and with development. However, the widths and center frequencies of the peaks seem quite stable, both with stimulus level (Fig. 7) and with age (Figs. 8 and 10). In contrast, Brown and colleagues have proposed that there is a single important amplitude maximum, which occurs when the emission frequency is a half-octave below f_2, i.e., when the emission frequency is about $0.7f_2$ (Brown, 1987; Brown and Gaskill, 1990a, b; Brown *et al.*, 1992, 1993; Brown and Kemp, 1985; A. M. Brown *et al.*, 1994; Brown and Williams, 1993; Gaskill and Brown, 1990). The stimulus levels usually employed in their recent measurements are $L_1 \times L_2 = 55 \times 40$ dB SPL, and they note that the half-octave relationship is ''lost'' if higher L_2 levels are allowed, but only for $2f_1 - f_2$ (Brown and Williams, 1993). While some of our gerbil data would fit with the half octave proposal, clearly most of our observed responses are more complicated. For example, peaks at an emission frequency a full octave below f_2 are found frequently and prominently, as well as peaks near $0.60f_2$. These differences appear real, and seem relatively unchanged as a function of stimulus amplitude. We certainly find these peaks for $L_1 \times L_2 = 50 \times 40$ dB SPL, for example.

Some of the differences between our results and those of Brown's group may be species differences, in that the majority of the data supporting their conclusions are drawn from humans and guinea pigs. In contrast, their gerbil observations (Brown and Kemp, 1985, Fig. 1) do show for $f_2 = 4$ kHz a prominent peak located at about $0.62\ f_2$, which persists at high stimulus levels and is joined by a lower-frequency peak. Further, a close examination of their human data shows that there often appear to be multiple peaks, including peaks at frequencies lower than a half octave below f_2 (although not as low as a full octave below). While it is a useful first approximation, therefore, the observed emission amplitude responses appear to be more complicated than the ''half octave'' rule can encompass, at least in gerbils.

D. The interpretation of the amplitude response

The structure in the amplitude response has been interpreted as evidence in favor of a ''second filter'' in the co-

chlea (Allen and Fahey, 1993a, b; Brown and Williams, 1993). The general idea is that distortion forces are generated in the outer hair cells (OHCs). In this model, the force generation is presumed to be relatively insensitive to stimulus frequency ratio, that is, insensitive on the scale of the amplitude structure, which involves changes in f_1/f_2 of 0.10 or less. At low stimulus levels, the maximum of distortion generation is assumed to occur in the OHCs located near the f_2 place. To be observed in the ear canal, these distortion forces must be translated into BM motion. It is in this translation that the emissions are assumed filtered by the micromechanical structure of the cochlea. One specific model has suggested that the filtering occurs in the tectorial membrane–outer hair cell stereocila system, which causes a ''short-circuit'' for distortion frequencies a half octave below f_2 (Allen and Fahey, 1993a).

This specific model now seems unlikely to be adequate, given recent results that show similar filtering in barn owls, and in certain alligator lizards who do not even have a tectorial membrane in the relevant frequency range (Tashenberger *et al.*, 1995). We may further note that the reported responses in these two very different species are remarkably similar to each other, and fairly similar to those in the gerbil reported here. The shape of the amplitude response is insensitive to stimulus level for both barn owl and lizard, over the limited range reported. There are apparently multiple peaks in both, for the barn owl at emission frequencies of $0.50f_2$ and $0.66f_2$ for f_2=7.9 kHz, and for the lizard at $0.43f_2$ and $0.70f_2$ for f_2=4 kHz. The peak frequencies noted above for the gerbil span these values.

Hearing in the gerbil does not begin until 13–14 days after birth (dab), and the endocochlear potential does not reach adult levels until after 20 dab. There are still structural changes involving the tectorial membrane through the early period, as well as possible BM changes associated with the place code shift (Schweitzer *et al.*, 1996). Further, there are significant changes in cochlear amplifier operation and base cutoff frequency through this period (Mills *et al.*, 1994; Mills and Rubel, 1996). In contrast, we have found in this study that the center frequencies and widths of the peaks in the emissions seem to be remarkably stable through most of this developmental period. Further, both amplitude and phase variations are generally the *same* for the active processes and the passive responses. This implies that, whatever the cause of the ''filtering'' of the emissions seen here, it is insensitive to specific details of the developing micromechanical BM structure. In short, the filtering itself does not appear to be associated with the operation of the amplifier operation. The generally increased overall amplitude of the emissions at low stimulus levels in mammals, of course, is directly attributable to the cochlear amplifier operation.

These results all argue that the structure in the emission amplitude response is unlikely to be a useful indicator of the tuning of the BM. Indeed, studies which have attempted to directly relate structure to tuning have so far found little or only moderately significant correlations (Brown *et al.*, 1993). The origin of the emission amplitude structure remains unknown.

It also should be noted that the structure discussed here is different from the ''threshold microstructure'' which is well known in humans (e.g., Long and Tubis, 1988a, b), and the fine structure in distortion product emissions recently demonstrated in humans (He and Schmiedt, 1993). Compared to the structure noted here, the threshold microstructure and emission fine structure both have a generally finer scale, are much more variable between individuals, and generally disappear with increasing stimulus level.

In contrast to the stable location in relative frequency of the amplitude peaks, mammals seem to have some variation in the ''envelope'' of the amplitude response as a function of age and frequency. That is, there is a variation in relative peak amplitudes with age. This variation may be related to the fact that an upper stimulus level limit for measurement of the ''half octave'' response in mammals has been reported for $2f_1-f_2$ (Brown and Gaskill, 1990a; Brown and Williams, 1993), e.g., this effect could be due to another component becoming dominant as the stimulus level increases. Here we suggest a modest change in relative peak amplitude in gerbils with age, stimulus level, and frequency. However, the envelope response seems of little practical use, since the emission amplitude response is so strongly dominated by the multiple peak structure.

We have also found significant developmental changes in the amplitude response at 2 kHz with age. This is in agreement with earlier studies in gerbils, showing that the cochlear amplifier in the apex continued to mature over these ages (Mills *et al.*, 1994; Mills and Rubel, 1996).

V. CONCLUSIONS

(1) Caution is indicated in interpreting signal delays from emission measurements. Because the variation of observed delay time depends strongly on stimulus level, a difference in the intrinsic passive threshold can cause an apparent change in signal delay. Conceptually, it makes more sense to base measurements on stimulus levels at a constant level relative to cochlear function, than relative to the amplitude in the ear canal. The procedure used here, to normalize the stimulus levels to the active–passive crossover level, appears promising but its validity remains to be independently established.

(2) Even with this correction, the round trip signal delay measured using distortion product emissions is not an adequate method of estimating physical distances in the cochlea, e.g., the distance to the place where the traveling wave peaks. Instead, the signal delay appears to depend primarily on the phase buildup of the traveling wave to the region in the cochlea where the emissions are generated. The considerable variation of the measured delay with stimulus amplitude (probably due to saturation effects in the cochlear amplifier) makes it impossible to accurately determine the contribution of any other delays.

(3) The presence of multiple peaks in the active emission is remarkably stable with age in both the emission frequencies at the amplitude maxima and the peak half widths, and is similar to that reported in birds and lizards. In contrast to these nonmammalian species, there appears to be a modest change in the relative magnitudes of the peaks in the $2f_1-f_2$ emission with stimulus intensity. Peaks at higher

emission frequencies (i.e., closer to f_2) appear to be relatively more prominent in younger animals.

(4) The cochlear amplifier at the base of the cochlea appears to be quite mature from the onset of hearing, in the aspects responsible for signal delay. This is in agreement with earlier studies (Mills *et al.*, 1994; Mills and Rubel, 1996), which found that the limitation on auditory function in the base of the cochlea stemmed from immaturity in the passive response. In contrast, significant development of cochlear amplifier function itself was noted for f_2 frequencies of 1–2 kHz. This delayed apical development is supported by the larger changes observed here with age in the amplitude structure at 2 kHz.

(5) There is an interesting minimum in the derived signal velocity which occurs first at low frequencies, then later at high frequencies, typically a few days to a week after the emission first becomes measurable at that frequency. The significance of this change is unknown.

(6) The pre- and post-injection emissions show the same amplitude and phase responses at similar stimulus levels. The simplest hypothesis to account for this observation is that they are produced by the same mechanism, emitted at the same place in the cochlea, and if ''filtered,'' filtered by the same process after production. Remarkably, the active emissions, those odd-order emissions at low stimulus levels which are interrupted by furosemide injection, also have the same basic structure as the high-level pre- and post-injection emissions. The active signal delays are generally larger than that for the passive emissions, which agrees with simple ideas about the effect of the cochlear amplifier on the signal delay. There is a rapid variation in the derived signal delay at the crossover between active and passive levels.

ACKNOWLEDGMENTS

We thank Brandon Warren for assistance with software development on this project, and two anonymous reviewers for constructive comments on an earlier version of the manuscript. Support was provided by Grant No. NIH DC 00395 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

Allen, J. B., and Fahey, P. F. (**1993a**). ''Evidence for a second cochlear map,'' in *Proceedings of the International Symposium on Biophysics of Hair Cell Sensory Systems*, edited by H. Duifhuis, J. W. Horst, P. van Dijk, and S. M. van Netten (World Scientific, River Edge, NJ), pp. 296–303.

Allen, J. B., and Fahey, P. F. (**1993b**). ''A second cochlear-frequency map that correlates distortion product and neural tuning measurements,'' J. Acoust. Soc. Am. **94,** 809–816.

Arjmand, E., Harris, D., and Dallos, P. (**1988**). ''Developmental changes in frequency mapping of the gerbil cochlea: Comparison of two cochlear locations,'' Hear. Res. **32**, 93–96.

Brown, A. M. (**1987**). ''Acoustic distortion from rodent ears: A comparison of responses from rats, guinea pigs, and gerbils,'' Hear. Res. **31**, 25–38.

Brown, A. M. (**1993**). ''Distortion in the cochlea: Acoustic $f_2 - f_1$ at low stimulus levels,'' Hear. Res. **70**, 160–166.

Brown, A. M., and Gaskill, S. A. (**1990a**). ''Can basilar membrane tuning be inferred from distortion measurement?'' in *The Mechanics and Biophysics of Hearing: Proceedings of a Conference held at the University of Wisconsin, Madison, WI, June 25–29, 1990*, edited by P. Dallos, C. D. Geisler, J. W. Mathews, M. A. Ruggero, and C. R. Steele (Springer-Verlag, New York), pp. 164–169.

Brown, A. M., and Gaskill, S. A. (**1990b**). ''Measurement of acoustic distortion reveals underlying similarities between human and rodent mechanical responses,'' J. Acoust. Soc. Am. **88,** 840–849.

Brown, A. M., Gaskill, S. A., Carlyon, R. P., and Williams, D. M. (**1993**). ''Acoustic distortion as a measure of frequency selectivity: Relation to psychophysical equivalent rectangular bandwidth,'' J. Acoust. Soc. Am. **93,** 3291–3297.

Brown, A. M., and Kemp, D. T. (**1984**). ''Suppressibility of the $2f_1 - f_2$ stimulated acoustic emissions in gerbil and man,'' Hear. Res. **13**, 29–37.

Brown, A. M., and Kemp, D. T. (**1985**). ''Intermodulation distortion in the cochlea: Could basal vibration be the major cause of round window CM distortion?'' Hear. Res. **19**, 191–198.

Brown, A. M., Gaskill, S. A., and Williams, D. M. (**1992**). ''Mechanical filtering of sound in the inner ear,'' Proc. R. Soc. London Biol. **250**, 29–34.

Brown, A. M., and Williams, D. M. (**1993**). ''A second filter in the cochlea,'' in *Proceedings of the International Symposium on Biophysics of Hair Cell Sensory Systems*, edited by H. Duifhuis W. Horst, P. van Dijk, and S. M. van Netten (World Scientific, River Edge, NJ), pp. 72–77.

Brown, A. M., Sheppard, S. L., and Russell, P. T. (**1994**). ''Differences between neonate and adult cochlear mechanical responses,'' Aud. Neurosci. **1**, 169–181.

Brown, D., Kimberley, B., and Eggermont, J. (**1994**). ''Cochlear traveling-wave delays estimated by distortion-product emissions in normal hearing adults and term-born neonates,'' J. Otolaryngol. **23**, 234–238.

Cohen, Y. E., Doan, D. E., Rubin, D. M., and Saunders, J. C. (**1993**). ''Middle-ear development V: Development of umbo sensitivity in the gerbil,'' Am. J. Otolarygol. **14**, 191–198.

Eggermont, J. J. (**1979**). ''Narrow-band AP latencies in normal and recruiting human ears,'' J. Acoust. Soc. Am. **65,** 463–470.

Fahey, P. F., and Allen, J. B. (**1986**). ''Characterization of the cubic intermodulation distortion products in the cat external auditory meatus,'' in *Peripheral Auditory Mechanisms: Proceedings of a Conference Held at Boston University, Boston, MA, August 13–16, 1985*, edited by J. B. Allen, J. L. Hall, A. Hubbard, S. T. Neely, and A. Tubis (Springer-Verlag, New York), pp. 314–321.

Gaskill, S. A., and Brown, A. M. (**1990**). ''The behavior of the acoustic distortion product, $2f_1 - f_2$, from the human ear and its relation to auditory sensitivity,'' J. Acoust. Soc. Am. **88,** 821–839.

Goldstein, J. L. (**1967**). ''Auditory nonlinearity,'' J. Acoust. Soc. Am. **41,** 676–689.

Harris, D., and Dallos, P. (**1984**). ''Ontogenic changes in frequency mapping in a mammalian ear,'' Science **225,** 741–743.

He, N.-j., and Schmiedt, R. A. (**1993**). ''Fine structure of the $2f_1 - f_2$ acoustic distortion product: Changes with primary level,'' J. Acoust. Soc. Am. **94,** 2659–2669.

Johnstone, B. M., Patuzzi, R., and Yates, G. K. (**1986**). ''Basilar membrane measurements and the traveling wave,'' Hear. Res. **22**, 147–153.

Keefe, D. H., Bulen, J. C., Arehart, K. H., and Burns, E. M. (**1993**). ''Ear-canal impedance and reflection coefficient in human infants and adults,'' J. Acoust. Soc. Am. **94,** 2617–2638.

Kimberley, B. P., Brown, D. K., and Eggermont, J. J. (**1993**). ''Measuring human cochlear traveling wave delay using distortion product emission phase responses,'' J. Acoust. Soc. Am. **94,** 1343–1350.

Kummer, P., Janssen, T., and Arnold, W. (**1995**). ''Suppression turning characteristics of the $2f_1 - f_2$ distortion-product otoacoustic emission in humans,'' J. Acoust. Soc. Am. **98,** 197–210.

Long, G. R., and Tubis, A. (**1988a**). ''Investigations into the nature of the association between threshold microstructure and otoacoustic emissions,'' Hear. Res. **36**, 125–138.

Long, G. R., and Tubis, A. (**1988b**). ''Modification of spontaneous and evoked otoacoustic emissions and associated psychoacoustic microstructure by aspirin consumption,'' J. Acoust. Soc. Am. **84,** 1343–1353.

Mahoney, C. F. O., and Kemp, D. T. (**1995**). ''Distortion product otoacoustic emission delay measurement in human ears,'' J. Acoust. Soc. Am. **97,** 3721–3735.

Martin, G. K., Lonsbury-Martin, B. L., Probst, R., Scheinin, S. A., and Coats, A. C. (**1987**). ''Acoustic distortion products in rabbit ear canal. II. Sites of origin revealed by suppression contours and pure-tone exposures,'' Hear. Res. **28**, 191–208.

Norton, S. J., Bargones, J. Y., and Rubel, E. W (**1991**). ''Development of otoacoustic emissions in gerbil: Evidence for micromechanical changes underlying development of the place code,'' Hear. Res. **51**, 73–92.

Mills, D. M., Norton, S. J., and Rubel, E. W (**1993**). ''Vulnerability and

adaptation of distortion product otoacoustic emissions to endocochlear potential variation,'' J. Acoust. Soc. Am. **94,** 2108–2122.
Mills, D. M., Norton, S. J., and Rubel, E. W (**1994**). ''Development of active and passive mechanics in the mammalian cochlea,'' Auditory Neurosci. **1**, 77–99.
Mills, D. M., and Rubel, E. W (**1994**). ''Variation of distortion product otoacoustic emissions with furosemide injection,'' Hear. Res. **77**, 183–199.
Mills, D. M., and Rubel, E. W (**1996**). ''Development of the cochlear amplifier,'' J. Acoust. Soc. Am. **100,** 428–441.
Norton, S. J., and Rubel, E. W (**1990**). ''Active and passive ADP components in mammalian and avian ears,'' in *The Mechanics and Biophysics of Hearing: Proceedings of a Conference Held at the University of Wisconsin, Madison, WI, June 25–29, 1990*, edited by P. Dallos, C. D. Geisler, J. W. Mathews, M. A. Ruggero, and C. R. Steele (Springer-Verlag, New York), pp. 219–227.
Plonsey, R., and Collin, R. E. (**1961**). *Principles and Applications of Electromagnetic Fields* (McGraw-Hill, New York).
Robles, L., Ruggero, M. A., and Rich, N. C. (**1986**). ''Basilar membrane mechanics at the base of the chincilla cochea. I. Input-output functions, tuning curves, and response phases,'' J. Acoust. Soc. Am. **80,** 1364–1374.
Rubel, E. W (**1978**). ''Ontogeny of structure and function in the vertebrate auditory system,'' in *Handbook of Sensory Physiology*, edited by M. Jacobson (Springer-Verlag, New York), pp. 135–237.
Rubel, E. W, and Ryals, B. M. (**1983**). ''Development of the place principle: Acoustic trauma,'' Science **219,** 512–514.
Rübsamen, R., Mills, D. M., and Rubel, E. W (**1995**). ''Effects of furosemide on distortion product otoacoustic emissions and on neuronal responses in the anteroventral cochlear nucleus,'' J. Neurophys. **74**, 1628–1638.
Sanes, D. H., Merickel, M., and Rubel, E. W (**1989**). ''Evidence for an alteration of the tonotopic map in the gerbil cochlea during development,'' J. Comp. Neurol. **279,** 436–444.
Schweitzer, L., Lutz, C., Hobbs, M., and Weaver, S. P. (**1996**). ''Anatomical correlates of the passive properties underlying the developmental shift in the frequency map of the mammalian cochlea,'' Hear. Res. **97**, 84–94.
Tashenberger, G., Gallo, L., and Manley, G. A. (**1995**). ''Filtering of distortion-product otoacoustic emissions in the inner ear of birds and lizards,'' Hear. Res. **91**, 87–92.
Walsh, E. J., and Romand, R. (**1992**). ''Functional development of the cochlea and the cochlear nerve,'' in *Development of Auditory and Vestibular Systems II*, edited by R. Romand (Elsevier, New York), pp. 161–219.
Whitehead, M. L., McCoy, M. J., Lonsbury-Martin, B. L., and Martin, G. K. (**1995a**). ''Dependence of distortion-product otoacoustic emissions on primary levels in normal and impaired ears. I. Effects of decreasing L_2 below L_1,'' J. Acoust. Soc. Am. **97,** 2346–2358.
Whitehead, M. L., Stagner, B. B., McCoy, M. J., Lonsbury-Martin, B. L., and Martin, G. K. (**1995b**). ''Dependence of distortion-product otoacoustic emissions on primary levels in normal and impaired ears. II. Asymmetry in L_1,L_2 space,'' J. Acoust. Soc. Am. **97,** 2359–2377.
Woolf, N. K., and Ryan, A. F. (**1984**). ''The development of auditory function in the cochlea of the mongolian gerbil,'' Hear. Res. **13**, 277–283.
Woolf, N. K., Ryan, A. F., and Harris, J. P. (**1986**). ''Development of mammalian endocochlear potential: Normal ontogeny and effects of anoxia,'' Am. J. Physiol. **250,** R493–R498.
Yancey, C., and Dallos, P. (**1985**). ''Ontogenic changes in cochlear characteristic frequency at a basal turn location as reflected in the summating potential,'' Hear. Res. **18**, 189–195.
Zweig, G. (**1991**). ''Finding the impedance of the organ of Cŏrti,'' J. Acoust. Soc. Am. **89,** 1229–1254.