Development of the cochlear amplifier

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(Received 15 June 1995; accepted for publication 26 February 1996)

The development of the cochlear amplifier was examined in gerbils aged 14 days after birth (dab) to adult, for stimulus frequencies from 1 to 48 kHz. Distortion product otoacoustic emissions (DPOAEs) were employed to determine the characteristics of active emissions associated with cochlear amplifier operation. DPOAEs were also used to determine the characteristics of ''passive'' emissions remaining when the cochlear amplifier operation was interrupted by acute furosmide intoxication. The input–output functions of the passive emissions, and of the active emissions at low stimulus levels, could be approximated by parallel straight lines. The horizontal distance between these parallel lines, i.e., the increase in stimulus level required to obtain a passive emission amplitude equal to the active emission, is an estimate of the gain of the cochlear amplifier. Further, the lowest stimulus level at which active and passive emissions become approximately equal defines a passive threshold level. At 14 dab, the cochlear amplifier gain was already at adult levels for the midfrequencies (4–8 kHz), but no emissions were detected at the extremes (at 1 kHz, and 24 kHz and above). During the period over which the endocochlear potential (EP) is known to increase most sharply (14 to 18 dab), the gain at all frequencies increased. At low frequencies there was little or no gain in the youngest age group, but matured by 23 dab. The gain at the middle frequencies subsequently decreased, resulting by 30 dab in a gain that was remarkably flat across frequency from 1 to 32 kHz. The passive thresholds generally improved with age at all frequencies, but most dramatically at the high frequencies. Results are consistent with the view that the elements of the cochlear amplifier are functional in the base of the cochlea at all ages, but that auditory function is primarily limited by the lower passive base cutoff frequency at younger ages. The proposed increase in passive base cutoff frequency with age accounts for the known place code shift.

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

INTRODUCTION

The cochlear amplifier is the name given to the collection of processes that cause physical amplification of traveling waves on the basilar membrane (BM; Davis, 1983). While the object of considerable research effort in recent years (Ashmore, 1987; reviews: Dallos, 1992; Patuzzi and Robertson, 1988) the precise mechanisms of operation of this amplifier remain uncertain. It is known that the amplifier saturates easily, therefore the amplification is most obvious at low sound levels. In fact, the response is approximately linear only at very low sound levels, above which the response is compressively nonlinear (Johnstone *et al.*, 1986; Ruggero and Rich, 1991). The amplifier itself is physiologically vulnerable and appears to depend on the presence and correct functioning of the outer hair cells.

It is also known that the amplifier operation is vulnerable to a sharp drop in the endocochlear potential (EP) caused by furosemide injection (Evans and Klinke, 1982; Kemp and Brown, 1984; Ruggero and Rich, 1990, 1991; Sewell, 1984a, b, c). Recently, we reported that the amplifier can, in fact, recover its full function in the presence of a persisting subnormal EP (Mills *et al.*, 1993; Mills and Rubel, 1994; Rübsamen *et al.*, 1995). This adaptive process was found to have a time scale of about 15 min. It was suggested that the primary function of the adaptive process is to provide a mechanism to adjust the gain of the cochlear amplifier so that it operates at or near its ''optimum'' value, which is presumed to be a function of stimulus frequency.

Most studies of the cochlear amplifier to date have been in adult subjects. It is expected that the study of the amplifier as it normally develops in younger subjects may be helpful in elucidating and constraining possible mechanisms of operation. For example, the presence of the adaptive processes implies the presence of a set point (the ''optimum'' value) for the gain at every frequency. While it is plausible to assume that these set points may change as the cochlear amplifier develops, it is not known if in fact they do so. The set point may also be a function of frequency. If so, changes in the set point may have an important role during development, when there are large changes in auditory function.

There are a number of other questions which may be addressed by careful study of the development of the cochlear amplifier. First, the discovery of the ability of the cochlear amplifier in the adult to recover full function with a subnormal EP (about 50 mV), at least at midfrequency ranges, leads us to question if the high mammalian EP is actually *necessary* for cochlear amplifier function. Evidence on this question can be obtained by studying cochlear amplifier function prior to development of an adult EP. Second, the high threshold of the earliest auditory responses suggest

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that the first cochlear response is essentially passive, but the cochlear amplifier could nonetheless be contributing significantly to auditory function at hearing onset. Finally, there is the place code shift known to occur in the base of the cochlea (Arjmand, 1988; Rubel, 1978; Rubel *et al.*, 1984; Rubel and Ryals, 1983; Rübsamen, 1992). The question has arisen whether this shift is essentially due to changes in passive basilar membrane properties, or to development of the cochlear amplifier, or to both (review: Walsh and Romand, 1992).

To answer these questions, it is most useful if the studies of the cochlear amplifier can encompass the time that the normal EP rises to mature values. In the Mongolian gerbil (*Meriones unguiculates*) the EP rises from about 25 mV at 14 days after birth (dab) to nearly adult values of 70 mV by 20 dab (McGuirt *et al.*, 1995; Woolf *et al.*, 1986). The middle ear typically clears of mesenchyme by 14 dab. There is therefore a substantial rise in EP in a short period, during which its impact on cochlear amplifier operation can be observed through a functional middle ear.

We have shown that distortion product otoacoustic emissions can provide a very useful, noninvasive method for investigating cochlear and peripheral functioning in developing gerbils (Norton *et al.*, 1991). We have also shown that the addition of a furosemide assay to these studies is useful for distinguishing between emissions which depend on the functioning of the cochlear amplifier and those which do not (Mills *et al.*, 1993; Mills and Rubel, 1994). The difference between the emission levels, pre- and postfurosemide, can be related to the gain of the cochlear amplifier at low stimulus levels. Our most recent study (Mills *et al.*, 1994) found that the cochlear amplifier was present at adult gain levels at midfrequencies in the youngest animals (15 dab) and that it rapidly developed at high and low frequencies with age. We also found that the *passive* properties of the cochlea were a significant limitation on hearing at high frequencies in the young animals.

These initial studies were, however, incomplete in several respects. First, equipment limitations prevented exploration of frequencies above 16 kHz. This is a serious limitation for gerbils, because the cochlear amplifier is functional above 16 kHz even in the youngest animals (Mills *et al.*, 1994), and hearing in adult animals extends to 60 kHz (Brown, 1973; Ryan, 1976). Measuring the properties of the cochlear amplifier at these higher frequencies is very desirable, both because such measurements are required to observe the effects of the base cutoff frequency (Puria and Allen, 1991) on cochlear amplifier operation, and because such high frequencies provide a more rigorous test of models of the cochlear amplifier (Santos-Sacchi, 1992).

Finally, the previous study established that the input–output function for the passive, postinjection emissions had a slope of about two. It was suspected that the input–output value for the normal, preinjection emissions might also tend to a similar slope if the measurements could be extended to sufficiently low stimulus levels. Among other benefits, such measurements would allow improved estimates of the cochlear amplifier gain.

Equipment has therefore been developed capable of making emission measurements for stimulus frequencies up to 50 kHz, with significantly reduced instrumental distortion. In the present study, we report results obtained in neonatal gerbils, using the furosemide assay that we previously developed to determine the characteristics of the cochlear amplifier (Mills *et al.*, 1993, 1994; Mills and Rubel, 1994; Rübsamen *et al.*, 1995).

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)	*N*	Furosemide (mg/kg)
14	7	60
16	4	80
18	8	100
23	5	120
29	5	150
42–46	5	300

I. METHODS

A. Animal preparation

Gerbils were raised in our colony from breeding pairs obtained from a commercial supplier (Tumblebrook Farms, Brookfield, MA). Cages were inspected once a day for births, and litters culled to six. The day on which the birth was noted was denoted 0 dab. (Note that this differs from the less conventional definition used in the previous report by Mills *et al.*, 1994, where dating began with 1 dab.) The numbers of experimental animals in each age group are given in Table I. Animal preparation and recording were performed in an IAC double-walled acoustic booth. Animals were initially anesthetized with a mixture of ketamine hydrochloride (Ketaset; 15 mg/kg) and xylazine (Rompun; 5 mg/kg). For young animals (14–23 dab) the initial injection was made subcutaneously, and anesthesia was maintained primarily by subsequent subcutaneous injections of ketamine alone (5–10 mg/kg). For older animals, the initial injection was by intraperitoneal (I.P.) injection, and anesthesia was maintained by subcutaneous injections of the ketamine–xylazine mixture at about one-third the initial dosage.

The pinna, surrounding skin and outer portion of the ear canal were removed on the left side, along with much of the scalp. The animal was fastened to a custom head bar with alpha cyanocrylate adhesive (Borden), and a thermocouple was inserted into the rectum. Internal temperature was maintained at 36–37 °C. Using an operating microscope, the external ear canal and tympanic membrane were inspected to insure that they were intact and clear. In about half of the 14 dab animals, but none of the older ones, mesenchymal fluid and debris were found behind the tympanic membrane. Emissions from these animals were never detectable above noise and instrumental distortion levels, and they were not included in the experimental group. A 1-mm hole was drilled in the bulla and a tube (0.6 mm i.d. by 18 cm) force-fitted into the hole to provide for static pressure relief between the middle ear and outer ear.

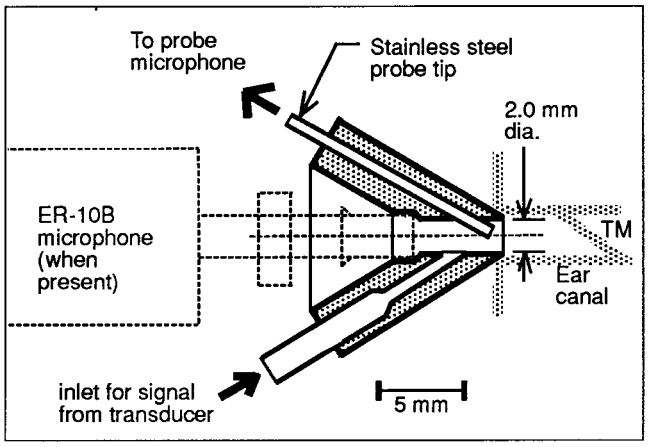

FIG. 1. Section view of one of two high-frequency couplers used for these measurements. The coupler is cone shaped overall, with a total opening angle of 66°. A 2.0-mm-diam hole through the center provides a cavity that couples the ear canal to an ER-10B microphone, a probe microphone, and two inlets for sound stimulus delivery. Only one inlet is shown, the other is rotated out of the section view. A brass support tube (not shown) is attached to a micromanipulator, with the z axis parallel to the 2.0-mm central bore.

B. High-frequency emission measurements

The basic computer system for generating the two stimulus tones and analyzing emissions was the same as that described previously (Mills *et al.*, 1993, 1994; Mills and Rubel, 1994). For the present experiments, however, two microphones were typically in place at the same time, both coupled to the ear canal with a high-frequency coupler (Fig. 1). The coupler illustrated had a 2.0-mm-diam hole forming a central cavity, which was connected to a sensitive, low noise microphone (ER-10B, Etymotic Research) and to a probe tube microphone. The probe microphone for this coupler consisted of a $\frac{1}{4}$-in. reference microphone (2530, Larson & Davis) connected to a 0.81-mm i.d.$\times$2.0-cm probe tube by a custom fitting. This probe tube microphone had previously been calibrated against the $\frac{1}{4}$-in. reference microphone using a substitution method. Briefly, this was done by first threading the $\frac{1}{4}$-in. microphone into a short tube with a sound source connected to the other end. A broadband signal was introduced, and the microphone response recorded. The microphone was replaced by an insert with the same outline as the microphone, but with a small hole through the center. The probe tube was inserted through this hole until the probe tip was flush with the surface of the insert. The same audio signal was introduced again, and the response of the $\frac{1}{4}$-in. microphone, now used as a probe microphone, was recorded. The ratio of the two responses was then stored in the computer, providing a correction of the probe microphone response to the $\frac{1}{4}$-in. reference microphone response. This system provided a usable response for stimulus frequencies up to 50 kHz.

For the high frequency coupler shown in Fig. 1, acoustic stimuli were generated by two tweeters (D19AD-05, Vifa) in custom enclosures, and were delivered to the coupler cavity through tubes connected to the two inlets, one of which is indicated in Fig. 1 (the other is rotated out of the plane of section).

For the youngest animals (14–16 dab), a smaller (1.4-mm-i.d.) coupler was used. This coupler used a proportionally smaller probe tube (0.5 mm i.d.) connected to an ER-7C probe tube microphone. This probe tube microphone, using a 2.5-cm-long probe tube, was also calibrated by replacement in a tube compared to the $\frac{1}{4}$-in. reference microphone. It provided a usable response to 35 kHz.

To fit either coupler to the ear canal, the ER-10B was temporarily removed, and the ear canal illuminated from the side with a fiber optic light source. Using an operating microscope, the ear canal was visualized through the central hole of the coupler while it was advanced with a micromanipulator. When a tight seal to the ear canal was obtained, the ER-10B was reinstalled in the coupler, as shown in Fig. 1. A wideband signal was introduced into the ear canal for 2–5 s by one of the custom tweeters. The comparison of the output of the probe tube microphone to the ER-10B provided *in situ* calibration of the ER-10B, with its measured sound-pressure level referenced to the location of the tip of the probe tube. Typically, the output of the ER-10B was used for measurements of both stimuli and distortion product emissions to about 30 kHz, while the probe tube microphone was used for measurement of stimuli only at higher frequencies.

To reduce the instrumental distortion that had limited previous measurements of the weakest emissions (Mills *et al.*, 1994) a programmable low-pass filter (Frequency Devices 828L8EX-4) was placed following the ER-10B microphone preamp. In normal use, the stimulus amplitudes were first checked with the filter turned off, and the attenuators adjusted if necessary. Then, the filter corner frequency was set approximately equal to $2f_1-f_2$, and the filtered signal amplified further before being sent to the analog/digital (A/D) converter. With the stimulus amplitudes reduced by 40 dB and more in the filtered signal, there was a significant reduction in distortion introduced at the A/D converter level. Remaining instrumental distortion levels were estimated using a variety of ''cavities,'' including long, uniform tubes with I.D.s equal to typical ear canal diameters, and 14-dab gerbils with mesenchyme-filled middle ears. The noncochlear origin of the distortion products measured in the animals with mesenchyme-filled middle ears was confirmed by noting that there was no change whatever with furosemide injection or death of the animal. All measurements gave essentially the same result, that instrumental distortion was not significant except for stimulus intensities above 100 dB SPL at frequencies below 24 kHz. Measured instrumental distortion will be summarized later, with results (Fig. 3).

C. Procedures

After calibration, normal emission input–output, or ''growth'' functions were obtained. Procedures and parameters were chosen to optimize measurements to meet two goals: (1) the clear delineation of the shoulder, or saturation, region in the growth function, (2) determination of the growth function slope at the lowest stimulus levels possible. The ratio of the two stimulus frequencies, $f_1<f_2$, was always $f_2/f_1=1.28$ (except as noted), and the stimulus level L_1 was 10 dB above L_2. For each growth function, stimuli were increased in 5-dB steps from a minimum of 20 dB SPL

(for L_1) to an absolute maximum of 110 dB SPL. Normally, the maximum stimulus level used was $L_1 = 80$ dB SPL. If emissions were weak, maximum stimulus levels were increased until subsequent emission amplitudes measured at the lowest stimulus levels began to decrease significantly. This procedure established safe upper limits for the use of higher stimulus levels, which were necessary in younger animals at frequencies $\geqslant$16 kHz.

Growth functions were typically obtained for all f_2 frequencies from 1 up to 48 kHz, stepped at intervals of one octave or less. For the 14- and 16-dab neonates, f_2 frequencies were 1, 2, 4, 8, 12, 16, 20, 24, and 32 kHz. For older animals, frequencies were typically 1, 2, 4, 8, 16, 24, 32, 40, and 48 kHz. Averaging times were typically increased to 10 s at lower emission amplitudes, resulting in a noise floor of about -20 dB SPL up to a frequency of 14 kHz for the emission at $2f_1 - f_2$ (for which $f_2 = 25$ kHz), with the noise floor remaining below 0 dB SPL up to the $2f_1 - f_2$ emission frequency of 28 kHz (for which $f_2 = 50$ kHz).

Measurements of the normal emission growth functions typically took 15–30 min. Following the pre-injection measurements, the animals were given an I.P. injection of furosemide (Abbot Labs). Table I lists the dosage used at each age. Dosage was set according to preliminary measurements and previous studies (Mills *et al.*, 1994; Mills and Rubel, 1994) to meet the following criteria: The dosage would: (1) be sufficient to reduce the vulnerable emissions to a ''flat minimum'' for a period of 5–10 min in at least 4 of 5 animals; yet (2) in all animals allow at least a partial recovery of function by one half-hour post-injection. These criteria were set to provide enough time to characterize the passive emissions, measured during the time of complete interruption of the cochlear amplifier function. In all animals in the experimental group, the dosages employed resulted in an effective interruption of the cochlear amplifier, followed by a partial recovery, as judged by the effect on the vulnerable emissions. The dosages listed in Table I represent an increased dosage for the older animals over that previously employed (Mills *et al.*, 1994). While these were large dosages, especially for the adults, emissions in the adults nonetheless always recovered at least partially by one half hour after injection, and no obvious signs of excessive toxicity were observed. For example, there was no increase in mortality during these experiments, compared to similar experiments with much lower furosemide dosages (Mills and Rubel, 1994). Further, even at higher dosages, there appears no reason to change our earlier conclusion that the major effect of furosemide upon cochlear functioning is through the change in EP (Mills *et al.*, 1993).

After the furosemide injection, the emissions at several stimulus levels were measured repeatedly, every few seconds. These levels typically included $L_1 \times L_2 = 60 \times 50$ dB SPL at 8 kHz and 70×60 dB SPL at 32 kHz. (The same levels were also measured frequently while the pre- and post-injection growth functions were obtained.) Within 7–10 min after injection, behavior characteristic of a complete interruption of the cochlear amplifier was typically seen; that is, a sharp drop of the vulnerable emissions followed by a plateau (Mills and Rubel, 1994). During the plateau, the sequence of post-injection growth functions was obtained. These growth functions were taken for all the frequencies for which there had been detectable emissions in the pre-injection measurements, but only including the higher stimulus levels. This set typically took 7–8 mins to obtain. The emissions from the animal were typically monitored for an hour postinjection, to confirm that at least a partial recovery occurred.

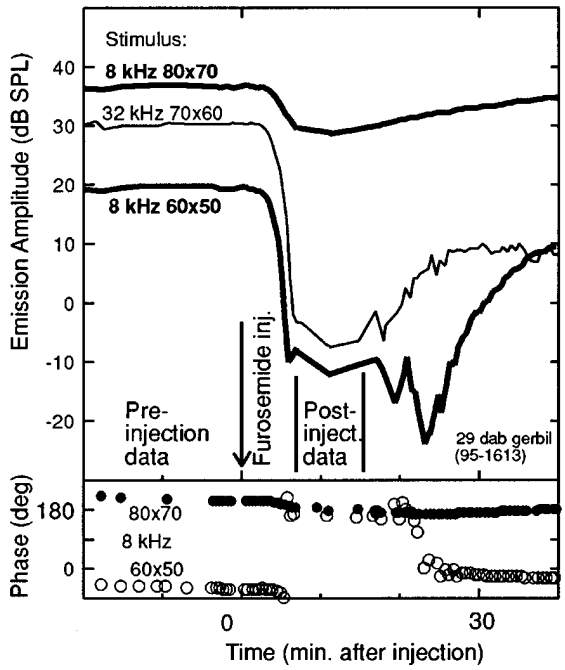

FIG. 2. Individual variation of cubic distortion tone (CDT) emissions with time after furosemide injection. The parameters noted are the stimulus parameters: The f_2 frequency followed by the stimulus levels $L_1 \times L_2$ in dB SPL. For all data, $f_2/f_1 = 1.28$, and L_1 is 10 dB above L_2. The upper panel displays the CDT amplitude response for three different stimuli, while the phase angle is displayed below, but only for the two 8-kHz stimuli. These data were from a 29-dab gerbil that received an I.P. furosemide injection of 150 mg/kg. Post-injection growth functions (such as shown in Fig. 3 and 4) were taken during the ''flat minimum,'' e.g., during the interval from 7.5 to 16 min noted in the figure.

We report here only the emission observed at the frequency $2f_1 - f_2$, also known as the cubic distortion tone (CDT) emission. While several other emission frequencies were also recorded routinely, our procedures throughout this experiment optimized the measurement of the CDT emissions. The results for the other emission frequencies confirm measurements reported earlier (Mills *et al.*, 1994) but add no essential new information.

II. RESULTS

A. Individual input–output functions

Typical variation with time of the monitored emissions is illustrated in Fig. 2 for a 29-dab gerbil. As shown, the emission amplitudes at high stimulus levels typically decreased less than 10 dB following furosemide injection, with only a small change in the associated phase angle. For lower stimulus levels, however, the emission amplitude dropped precipitously, with an associated large change in the phase angle. These emissions were then relatively constant at a low amplitude for a period of 10–15 min, after which there was a

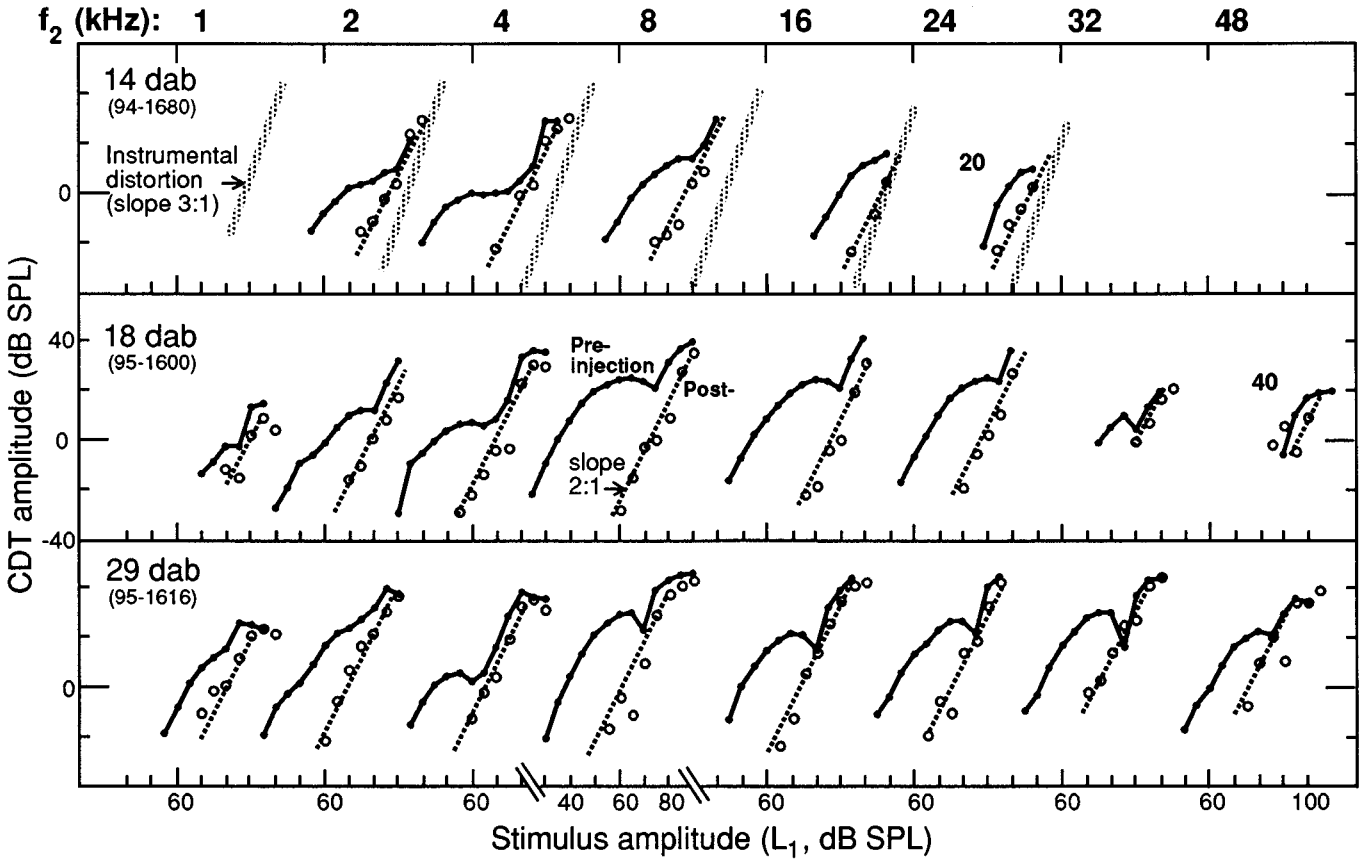

FIG. 3. Input–output or ''growth'' functions, showing the emission amplitudes versus stimulus levels for three different individual gerbils, for the f_2 frequencies noted along the top. The stimulus level for the lower frequency, L_1, is shown on the horizontal axis (note that the L_2 stimulus levels are 10 dB lower). Preinjection data are shown by the solid lines, post-injection data by open circles. A dashed line with slope 2:1 is fitted (by eye) to the post-injection data for illustrative purposes. The measured instrumental distortion, which had a slope of 3:1, is shown with the data for the 14-dab animal. This distortion was the same for the other animals, but is not shown as it did not limit or interfere with measurements on older animals. Points near the noise floor have been omitted for clarity. There were no emissions above instrumental distortion or noise levels for the youngest animal (14 dab, top panel) for an f_2 frequency of 1 kHz, or for $f_2=24$ kHz and above. We substitute the measurements with $f_2=20$ kHz for the 24-kHz data for the 14 dab, and 40 kHz for the 48 kHz for the 18-dab animal, as noted. Also, note that the effective stimulus levels to produce measurable emissions for the younger animals are larger, so that the curves are farther to the right, especially at higher frequencies.

return of the phase angle to the pre-injection value and the emission amplitude showed a rapid, but partial, recovery. Note that at the higher furosemide dosages used here, the recovery was typically not as complete as was seen earlier with smaller dosages (Mills *et al.*, 1993; Mills and Rubel, 1994).

The amplitude decrease illustrated in Fig. 2 for $f_2=32$ kHz was very similar to that at 8 kHz, occurring at the same time and having about the same magnitude decrease. However, the emission at the highest frequencies usually recovered somewhat earlier, as shown. Because of the more rapid recovery, the post-injection growth functions were first obtained for the higher frequency emissions, and those for $f_2=1$ to 4 kHz were obtained last. All of the data were obtained during the ''flat minimum'' period illustrated in Fig. 2.

Typical individual growth functions obtained pre- and post-injection for animals of three different ages are presented in Fig. 3. The pre-injection functions are shown by the solid lines, the post-injection data by the open circles. The measured instrumental distortion is also illustrated along with the 14-dab data. Note that the slope of the instrumental distortion input–output function at $2f_1-f_2$ is 3:1. No instrumental distortion could be measured above the noise floor at f_2 frequencies of 24 kHz and above. The instrumental distortion was the same for other ages, but only provided a constraint to the measurements at the youngest age studied, and only at the highest stimulus levels.

For the 14-dab animals at $f_2=1$ kHz, there was typically no emission found above noise and instrumental distortion levels, as illustrated. In contrast, the emissions for $f_2=4$ kHz were typically very adultlike, as shown. However, the emissions for f_2 frequencies above 4 kHz at 14 dab typically required significantly greater stimulus levels than in adults to be measurable. Further, at the highest frequencies for which emissions were detected, the pre- and post-injection emissions were typically not very different. For the 14-dab animal shown, for example, the highest frequency for which any emission was measurable above the noise floor was at $f_2=20$ kHz, as indicated in the top panel of Fig. 3.

As the animals aged, the emissions improved rapidly at both high and low frequencies. For the 18-dab animal shown, for example, the maximum f_2 frequency for which emissions could be detected had increased to 40 kHz (i.e., no emission

was detected above the noise floor for $f_2=48$ kHz). For this animal, there was a clear difference between pre- and postinjection emissions at 32 kHz, but not at 40 kHz. More usually, the difference was small, but obvious, even at the highest frequencies for which emissions could be obtained.

By 29 dab, adult-like responses were typically found at all frequencies, as illustrated in the example. There were emissions found at both extremes, for $f_2=1$ and 48 kHz.

In contrast to the development of active responses, the post-injection, passive responses were very similar in form at all ages and frequencies. Below obvious saturation effects at the highest intensities, the post-injection input output functions could usually be fitted quite well with a straight line with a slope of about 2:1. In Fig. 3, dashed lines with slopes of exactly 2:1 are shown for illustration.

Linear, least-squares fits were made to the post-injection responses at moderate stimulus levels, that is, ignoring data affected by obvious saturation at the highest stimulus levels and noise at very low stimulus levels. The resulting means and standard errors are shown in Fig. 4. It is clear that across animals and f_2 frequencies the passive slope was generally very close to 2:1. For all f_2 frequencies above 2 kHz, there was a tendency for the slope to increase with age. The slope typically reached adult levels several days to a week after emissions could first be detected at that frequency. The mean passive slopes ranged from a low value of 1.7 at $f_2=8$ kHz for 14 dab animals to a high of 2.4 for $f_2=4$ kHz in adult animals.

As illustrated in Fig. 3, the pre-injection growth functions also tended to a slope of *approximately* 2:1 at the lowest stimulus levels, at all frequencies and ages. That is, the normal ''active'' CDT emission typically grew from the lowest stimulus levels with a slope of about 2:1, then the slope slowly decreased until the curve flattened completely or even showed a sharp decrease and recovery, or a ''notch.'' For many animals, at many frequencies, a slope of about 2:1 at even moderate stimulus levels was quite obvious, e.g., the response of the 18-dab animal at 24 kHz, or the 14 dab at 16 kHz (Fig. 3). In some cases, however, the slope decreased gradually as the stimulus intensity decreased, only reaching a slope of approximately 2:1 at the lowest stimulus levels, e.g., the 18-dab subject at 8 and 16 kHz in Fig. 3, or for the 14-dab animal at 4 kHz. In some animals, the signal-to-noise ratio was too poor to reach this slope (e.g., 14 dab at 2 kHz) and the most that could be said was that the response was not inconsistent with an eventual slope of about 2:1. For these reasons, it could also not be established with certainty that the slope of the active component at low signal levels *in all cases* became parallel with the slope of the passive component. As another complication, there appeared to be several active components at 2 kHz in older animals, each showing modest saturation at different stimulus levels, as illustrated for the 29 dab animal in Fig. 3.

At the highest stimulus levels, the CDT response was typically changed little by furosemide intoxication, and can be considered essentially passive, as the examples in Fig. 3 illustrate.

The approximate equality of the pre-injection slope at low intensities to the measured postinjection slope at all but the highest intensities allows a unique measure of the cochlear amplifier gain, as illustrated in Fig. 5. This is the horizontal distance, A_c, between the two parallel lines which represent the active and passive responses extrapolated to very low signal levels. Consider the use of any arbitrary emission ''threshold'' measure, such as the stimulus level, L_p, required to obtain a passive emission amplitude equal to 0 dB SPL. Now consider the improvement in this threshold with the cochlear amplifier operative, the dimension A_c in Fig. 5. Because there is no change in stimulus and emission frequencies between these two growth functions, the threshold improvement A_c must be solely due to the action of the cochlear amplifier. The dimension A_c (in dB) can therefore be taken as an estimate of the gain of the cochlear amplifier.

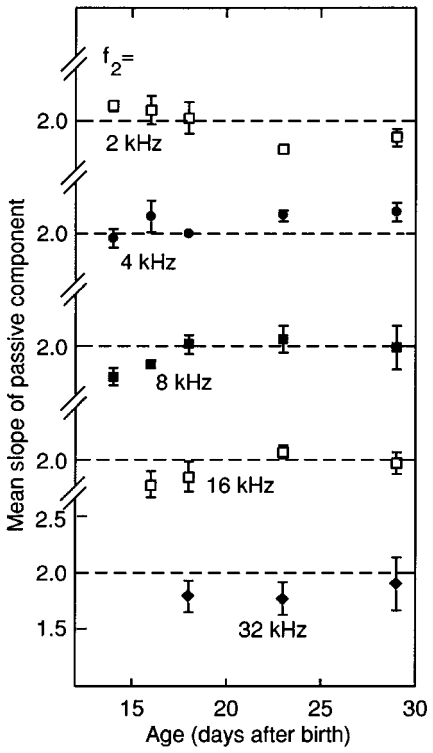

FIG. 4. Group data. The slope of the post-injection cubic distortion tone emission growth function at moderate stimulus levels as a function of age for f_2 frequencies from 2 to 32 kHz. For each frequency, the horizontal dashed lines represent a slope of 2.0. Derived slopes are those for linear, least squares fits to the data points at moderate stimulus levels, ignoring data points affected by obvious saturation effects at the highest stimulus levels and noise at the lower stimulus levels. Bars indicate standard errors of the mean. Numbers of animals in each age group are listed in Table I.

At moderate to high stimulus levels, the saturation of the active processes usually resulted in a ''shoulder'' as seen in Fig. 5. The extrapolation of the shoulder to the post-injection response defines the stimulus level, L_x, required to reach the normal active–passive transition. Below this ''crossover'' stimulus level, the normal CDT response is dominated by active processes, but above the crossover level the response is dominated by passive processes. Both L_x and L_p can be considered threshold measures, although with different meanings, for the passive, post-injection response. Further, the quantities (L_p-A_c) and (L_x-A_c) both represent corresponding thresholds of the *combined* active and passive re-

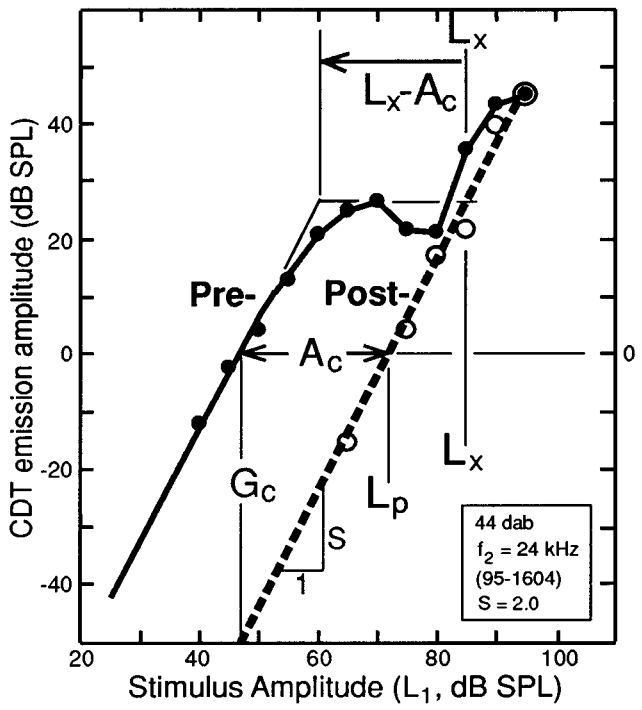

FIG. 5. Typical growth function and definitions for analysis of the cubic distortion tone (CDT) emission. The pre-injection function is shown by the solid line. The ''post-injection'' amplitudes, taken during the period when emissions decreased to a flat minimum after furosemide injection (Fig. 2) are shown by open circles. A line with slope S is fitted to the post-injection data as shown. The vertical difference (in dB) between the pre- and post-injection growth functions at low stimulus levels, where they are parallel, is denoted G_c, and the horizontal distance is A_c. Obviously, $A_c = G_c/S$. Extrapolation of the preinjection shoulder to the post-injection, ''passive'' response defines the stimulus level, L_x. Above L_x the passive component normally dominates the active, and below it the active response normally dominates the passive. The level L_x therefore defines a passive ''threshold'' measure which is the stimulus required to reach a criterion level in the cochlea, i.e., that stimulus level characteristic of the active–passive crossover. One can also define a ''combined'' threshold measure for the sum of the passive plus active component, which is $(L_x - A_c)$ as shown. Another passive threshold measure, L_p, can be defined as the stimulus level required for the postinjection CDT amplitude to reach 0 dB SPL, as illustrated. The example shown is from a young adult gerbil, 44 days after birth (dab) with $f_2 = 24$ kHz. The distance, A_c, is 23 dB SPL and this is the derived cochlear amplifier gain. The threshold measures are $L_x = 83$ dB SPL and $L_p = 71$ dB SPL, giving the combined response threshold, active plus passive, $(L_x - A_c)$ equal to 60 dB SPL.

sponses. Differences between L_p and L_x will be considered in the discussion.

As noted above, it was not always possible to unequivocally determine the slopes of the active and passive components as in Fig. 5. This was particularly a problem at the extremes, where the emission amplitudes were weak. However, the derived gains are relatively insensitive to the precise slope. For growth functions where it was possible to determine the slopes unequivocally, a comparison of the derived gain using the actual slope to that obtained using a slope of 2.0 showed that the estimate using the approximation (2.0) was in all cases within $\pm 10\%$ of the actual shift A_c. As a useful approximation for this report, therefore, the values of A_c were estimated using a ''model'' response with a slope of exactly 2:1, and the gain estimates and other parameters were determined by fitting the model to the data.

Because of time constraints on post-injection measurements, our derived values for gain and threshold measures in this report are based on measurements at specific ratios of the two stimulus frequencies and amplitudes, i.e., $f_2/f_1 = 1.28$ and $L_1/L_2 = 3.1$ (10 dB difference). However, it is well-known that, at any given set of stimulus levels, there is some variation in the magnitude of the emission produced as a function of the ratio of the stimulus frequencies, f_2/f_1 (e.g., Brown and Gaskill, 1990; Whitehead *et al.*, 1992). For example, in adult gerbils, the ratio of f_2/f_1 which gives the maximum stimulus amplitude decreases somewhat as the f_2 frequency increases (Mills and Rubel, 1994). It has been suggested that changes in the f_2/f_1 response occur during development (Brown *et al.*, 1995).

While the objectives of the present study did not include a detailed examination of changes in the response as a function of f_2/f_1 with age, such changes should nonetheless be considered. Therefore, for several additional individual animals, measurements were made at frequency ratios of 1.20, 1.24, 1.28, 1.32, and 1.36, at a limited selection of f_2 frequencies. Note that it is not the possible variations in absolute emission amplitude with frequency ratio that are important for this study, but possible variations in the *differences* between active and passive responses, i.e., in the derived cochlear amplifier gains. Gains were separately estimated from the growth functions for all five ratios, as described above. Typical results for individual animals at three different ages are shown in Fig. 6.

While there are some variations with frequency ratio, particularly at the youngest ages and low frequencies, it is obvious that the same developmental trends can be seen across frequency ratio. For example, at $f_2 = 2$ and 8 kHz, there was an increase in gain from 15 to 20 dab, which was then reduced again by 30 dab. (As we will see below, these individual trends were verified by the mean values.) Therefore, it does not seem likely that developmental changes in the derived values for gain and threshold at a given frequency ratio can be ascribed to changes over development of the effects of the f_2/f_1 ratio on emission amplitude.

B. Group data: Development of cochlear amplifier gain and passive thresholds

Values for the gain estimate, A_c, and both ''thresholds,'' L_p and L_x, were calculated at each frequency for each animal, and averaged within each age group (Table I). Figure 7 contains the basic results as plots of response versus frequency, with age as the parameter. When first observed at 14 dab, the cochlear amplifier gain was found to have a broad maximum at the middle frequencies, and was already at adult levels near $f_2 = 4$ kHz. The most obvious change over the first four days (upper panel of Fig. 7) was that the gain of the cochlear amplifier ''ballooned'' up, increasing at all frequencies, and increasing most at the higher frequencies. During this same period, the EP rises rapidly in the developing gerbil (Woolf *et al.*, 1986).

However, the EP reaches near adult values by about 20 dab. In contrast to its earlier behavior, the cochlear amplifier gain from 18 to 44 dab was marked by a steady *decrease* in gain at the middle frequencies. At this same time, there was

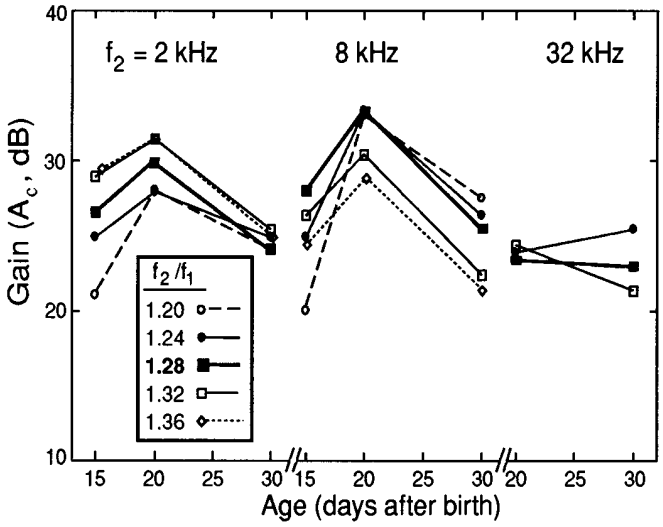

FIG. 6. Variation of the derived gain, A_c, with frequency ratio (f_2/f_1) for three individual animals, aged 15 days after birth (dab; animal #95-1629), 20 dab (#95-1634), and 30 dab (#95-1622). The parameter at the top is the f_2 frequency. The gains are derived from growth functions as shown in Fig. 5, with complete growth functions being obtained both pre- and post-injection for five f_2/f_1 ratios, at 1.20, 1.24, 1.28, 1.32, and 1.36 as indicated in the key. There were no emissions detected with $f_2=32$ kHz for the 15-dab animal, and even for the older animals the noise floor at 32 kHz precluded adequate measurements at the extreme frequency ratios. Note that data for all other figures in this report were obtained for $f_2/f_1=1.28$ only.

a significant increase in gain at low frequencies. This resulted in an adult cochlear amplifier gain which was quite flat across frequency. For example, for the frequency range from 2 to 32 kHz, the derived gain for the cochlear amplifier for 23 dab and older animals was flat to ± 3 dB, where at 16–18 dab it was 15 dB higher at the middle frequencies than at 2 and 32 kHz. By adulthood (42–46 dab) the gain even appeared to have a relative minimum near 4 kHz, so that it compensated partly for the fact that the passive threshold continued to have a peak in sensitivity near 4 kHz. These effects resulted in a combined gain ($A-L_x$) that was much flatter across frequency than it would be otherwise. Above 32 kHz, the gain consistently dropped very sharply, with a slope such that it appeared that the gain took slightly less than an octave to go from full gain to zero.

Figure 7 also displays the frequency variation for the stimulus level, L_x, characteristic of the active-passive crossover (defined in Fig. 5). This passive threshold measure is plotted so that lower thresholds (higher sensitivity) are at the top, for direct comparison with the gain plotted immediately below in each panel. At 14 dab, L_x had a sharp minimum near 4 kHz. As the animals developed, the thresholds at and below 4 kHz changed relatively little, while the thresholds at higher frequencies improved steadily. Adding in the changes in cochlear amplifier gain, this resulted in a combined threshold which was relatively flat across frequency in the adult gerbils.

We next (Fig. 8) compare directly the variation of gain with age found in this study to the variation of EP with age found by Woolf *et al.* (1986). At the higher frequencies (upper panel, Fig. 8) the gain increased sharply as the EP did, but after the EP stabilized, the gain was subsequently reduced. A uniform reduction then occurred over the 6 days from 23 to 29 dab.

The strong ''overshoot'' in gain found at mid- and high frequencies, however, did not occur at lower frequencies (lower panel). The gain at the lowest frequency (1 kHz) steadily improved as the EP rose. For the middle frequencies (4–8 kHz), in contrast, the gain was found to be already at adult levels at 14 dab, where the mean EP is only 25 mV.

The variation with age of the characteristic threshold levels (defined in Fig. 5) are presented in Fig. 9. For clarity, we plot the changes (in dB) relative to their values at 42–46 dab. Therefore, the value shown at any age is the *improvement* in threshold that occurs between that age and adulthood. The improvement was greatest from 14 to 18 dab, and at the higher frequencies. For example, there was nearly a 35-dB improvement in both threshold measures for $f_2=24$ kHz, from 17 dab to adulthood. In contrast, the thresholds at frequencies below 4 kHz improved much less. At 1 kHz, for example, L_x improved by only 6 dB over the same time period.

Note further that the two threshold measures, L_x and L_p, both improved steadily with age, and in an orderly fashion. No ''overshoots'' were found, as there were for the active gain functions. Also, the changes in L_p were typically somewhat larger than the changes in L_x. To quantify this relationship, these two threshold measures are plotted against each other in Fig. 10. For comparison, a reference line and the line marking the direction of maturation in Fig. 10 are drawn at a slope of 1.5. As will be shown in the discussion, this would be the slope expected if the change in threshold were entirely due to improvement in bidirectional passive conductance in middle ear and cochlea.

Finally, the combined threshold measure including both active and passive processes, L_x-A, is plotted in Fig. 11(a) so that the variation across age and frequency can be directly compared. There was a uniform, orderly improvement across frequency. This included an unusual feature, a relatively flat spot in the threshold between 24 and 32 kHz which was first seen at 16 dab. The improvement in threshold in this region was quite substantial from 16 to 29 dab, but the threshold remained quite flat. In contrast, there was little change in the combined response from 29 dab to 44, except for approximately a 10-dB improvement around 16 kHz.

For comparison, we also present in Fig. 11(b) results for neural thresholds in the gerbil ventral cochlear nucleus (Woolf and Ryan, 1985) and behavioral thresholds for adult gerbils (Ryan, 1976). The comparison will be discussed later.

III. DISCUSSION

Our analysis of the contributions of active and passive mechanics to the development of auditory responses requires consideration of the methods used, the assumptions underlying these methods, and the derived data. We first discuss the methods, then consider how the data obtained may add to our understanding of the relative contributions of active and passive mechanical properties to the ontogeny of cochlear function.

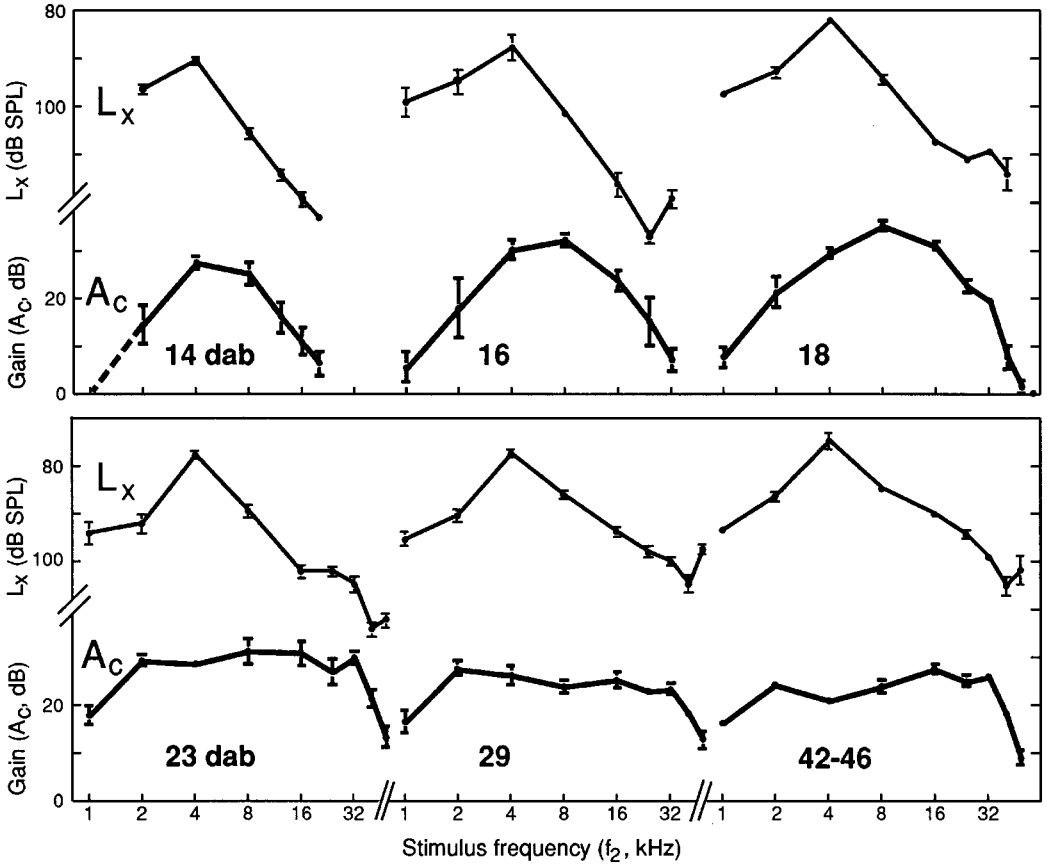

FIG. 7. Group data. Mean values of gain and threshold measure, averaged over each age group. The stimulus frequency, f_2, is plotted on the horizontal axis, with the gain, A_c, immediately above. The stimulus level characteristic of the active to passive crossover, L_x, is plotted above the gain. Note that, in this figure only, this threshold measure is plotted with an inverted axis for easier comparison with the gain measure, so that increased sensitivity is upward for both curves. Error bars indicate standard errors of the mean. See Fig. 5 for definitions. The parameter is the age in days after birth (dab).

A. Methodological and analytic issues

1. Estimating the gain of the cochlear amplifier

From Fig. 5, it is obvious that if the slope of the input–output function for the postfurosemide emission is the same as that for the normal emission at low stimulus levels, then it is possible to define a unique measure of the cochlear amplifier gain, the shift A_c. This is the horizontal distance between these two growth functions, where they are parallel. Because the only difference between these two growth functions is presumed due to the relative functionality of the cochlear amplifier, this shift must be related to the gain of the cochlear amplifier. For our purposes, the value A_c will be taken as an *estimate* of the cochlear amplifier gain. A formal equivalence might seem intuitively obvious, because A_c is the improvement in ''threshold'' observed with the cochlear amplifier functional compared to nonfunctional. However, while there is not space to do so here, reasonable examples can be constructed which demonstrate that while A_c is approximately equal, it is not *necessarily* exactly equal to the gain of the cochlear amplifier.

If the slopes of the two growth functions are equal, the unique vertical distance between the two curves, G_c, is equal to A_cS, where S is the slope of the growth function. Even if the slopes are not equal, or if the signal-to-noise ratio is such that the region of parallel slopes cannot be measured, the difference between these two curves can still be quantified. For example, in our previous work (Mills *et al.*, 1994) the gain of the cochlear amplifier was characterized by the ''gain figure'' G_{30}. This was defined as the (vertical) difference between active and passive emissions measured at a stimulus level 30 dB below the active-passive transition level, L_x. This measure was employed because the previous measurements did not extend to low enough stimulus levels to employ the quantities, G_c/S or A_c, used here. Clearly, G_{30} will always be less than or equal to G_c. In our earlier report, we argued that G_{30} would be *proportional* to the gain of the cochlear amplifier, not equal to it. From the discussion above, we now see that the constant of proportionality should be approximately two, for both G_c and G_{30}, for the parameters employed in this report. However, because the measurement of G_{30} does not extend to low enough stimulus levels, $G_{30}/2$ will tend to underestimate the actual gain at low stimulus levels, and it will probably not be as accurate a measure of the cochlear amplifier gain as the threshold shift, A_c, used in the present report.

2. The meaning of the characteristic stimulus levels L_p and L_x

We must also ask under what circumstances L_p and L_x will correctly estimate ''passive thresholds;'' that is, will be proportional to the response of the passive cochlear mechanics to sound levels in the ear canal. First, consider the

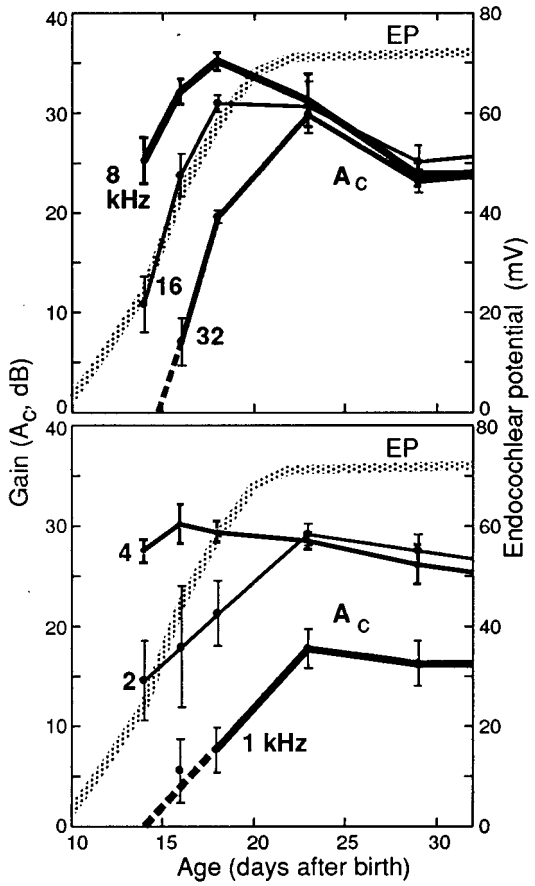

FIG. 8. The change of the mean gain, A_c, with age. The parameter listed is the f_2 frequency. Bars indicate standard errors of the mean. The dashed lines in the 1- and 32-kHz data indicate the probable variation, given that there were typically no emissions detected above noise and instrumental distortion levels at 14 dab at either of these frequencies. Same data as in Fig. 7. For comparison, the variation of the mean EP with age from Woolf *et al.* (1986) is also shown, with values on the right axis.

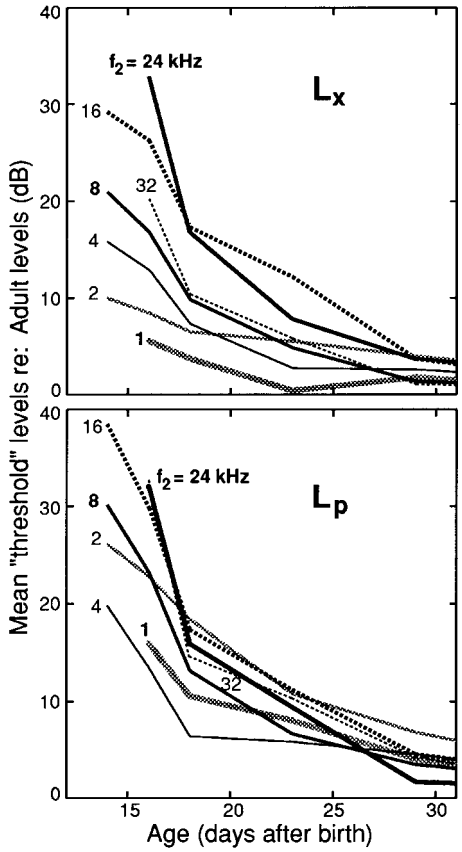

FIG. 9. Change of two passive threshold measures, L_x and L_p with age. The parameter is the f_2 frequency. For both, mean values are given, in dB, relative to those measured for the 42–46 dab age group. Therefore, the threshold value shown at any given age is the *improvement* in threshold that will occur between that age and adulthood. The stimulus level required to reach the active–passive transition, L_x, is displayed in the upper panel. The lower panel displays the level, L_p, required for a passive CDT emission of 0 dB SPL. See Fig. 5 for precise definitions. Note that the vertical axes are in the more usual direction for thresholds, and opposite to those in Fig. 7. Error bars are not shown for clarity.

active–passive transition level, L_x. For all stimulus levels higher than L_x, the response of the cochlea is dominated by the passive properties of the cochlea. Clearly, *if* this characteristic level at the point of emission generation in the cochlea does not change during development, L_x will depend only on the passive transmission of sound from the ear canal to the point of emission. That is, L_x would depend on passive input transmission properties only. Note that, under these circumstances, the interpretation of L_x as an input transmission threshold measure does not depend on the actual cochlear characteristic which allows its determination, only that such a response is characteristic *of the cochlea*.

By definition, L_p clearly depends only on passive characteristics (Fig. 5). However, unlike L_x it depends not only on the passive input transmission at frequencies f_1 and f_2 to the point where the emissions are generated, but on the amplitude of the emissions generated and on transmission *back out through the middle ear* at the frequency $2f_1-f_2$. To compare the two measures, note that if there were a conductive loss, of R dB, inserted in the peripheral path that was constant across frequency and bidirectional, then L_x would increase by R dB while L_p would increase by $(R+R/S)$ dB. The additional R/S increase for L_p is due to the fact that the stimulus level must be increased by R/S in order to compensate for the R loss in the reverse transmission (a linear passive input–output slope equal to S is assumed in the appropriate stimulus region). For $S\cong 2$, the increase in L_p would then be about $1.5R$.

It can be seen (Fig. 10) that the data generally fit the slope $L_p/L_x=1.5$. This leads to two tentative conclusions. One is that the major overall effect of development on the passive properties of the input transmission path is equivalent to an increase in conductivity which is approximately uniform across frequency and bidirectional. This conclusion is supported by a number of other measures of the peripheral system, which show that the effect of the middle ear itself during development in the gerbil is a *relatively* uniform conductive improvement across frequency from 14 dab on (Ryan and Woolf, 1992). As we will discuss below, this improvement is probably not only due to changes in the middle ear function, but involves changes in the entire passive input transmission path from outer ear to the place of emission generation in the cochlea.

The steady improvement with age, and lack of a minimum in either passive measure (Fig. 9) during the time in

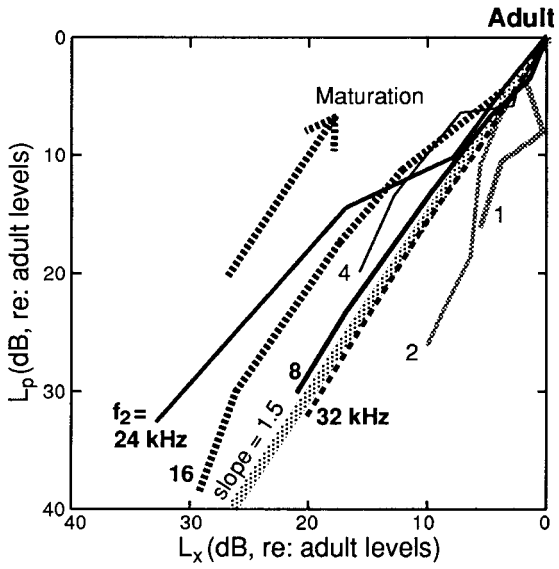

FIG. 10. Direct comparison of the mean stimulus levels for the active passive crossover level, L_x, with that for the passive threshold L_p. As in Fig. 9, both threshold measures are shown relative to their mean adult values at each frequency. The parameter listed is the f_2 frequency, and the direction of increasing age is shown. For comparison, the dashed lines show the slope of 1.5 for the ratio L_p/L_x (both in dB).

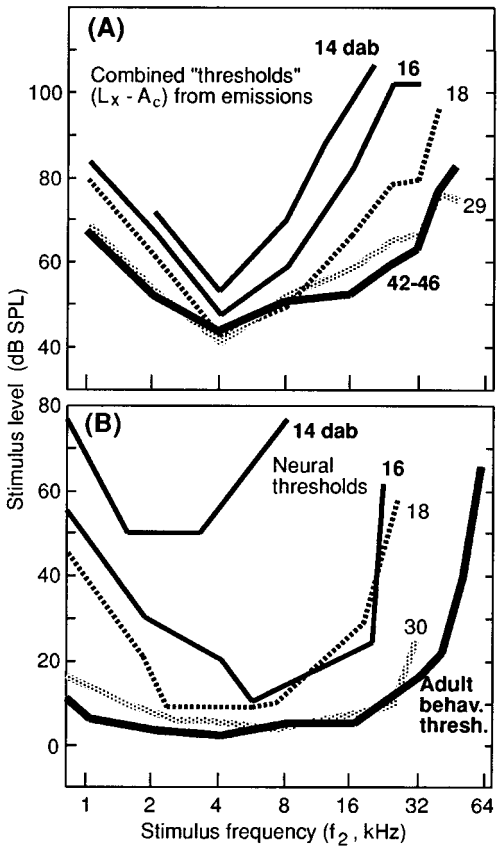

FIG. 11. (A) The variation of the mean combined threshold measure, $(L_x - A_c)$, with f_2 frequency. The parameter is age in days after birth (dab). (B) For comparison, we plot thresholds for neural responses in the ventral cochlear nucleus obtained by Ryan and Woolf (Fig. 3, 1985) for 14- to 30-dab animals. The curve marked ''Adult'' represents behavioral thresholds obtained by Ryan (Table I, 1976).

which the cochlear amplifier gain has a maximum (Fig. 8) provides additional modest evidence that these two quantities provide measures of what was hoped, that is, measures of passive properties of the cochlea and peripheral system.

B. Development of cochlear function

1. Effects of finite base cutoff frequency

We have previously argued that a finite base cutoff frequency will affect both the passive and active responses (Mills *et al.*, 1994). In this context, the base cutoff frequency, f_p, is defined to be the frequency above which passive waves do not propagate along the BM. Precisely, f_p is the lowest frequency at which the maximum passive response (the passive best frequency) is located at the extreme basal end of the cochlea. The simple model previously presented (Mills *et al.*, 1994) predicts that there will be a relative increase in the passive threshold even for frequencies *below* f_p because stimuli at these frequencies do not receive full benefit of the passive growth of waves along the BM. We also pointed out that, *if* it is true that the active process starts to amplify BM waves only when they reach the frequency/place corresponding to the passive maximum (Johnstone *et al.*, 1986), then the active process will not be affected by the base cutoff until the stimulus frequency is equal to f_p. Further, if the active amplification region is one half octave wide, as has been suggested (Brass and Kemp, 1993; Johnstone *et al.*, 1986), the gain of the cochlear amplifier would be expected to fall quite sharply for frequencies above f_p, reaching zero in one half octave (Mills *et al.*, 1994).

The present results substantially support the simple model which was presented earlier (Mills *et al.*, 1994) with minor modifications. In the adult gerbil (see 42–46 dab in Fig. 7) f_p appears to be approximately 32 kHz. As the simple model predicts, there is a steep increase in the passive threshold as the stimulus frequency approaches 32 kHz, then a relatively flat threshold for frequencies above f_p. In contrast, the active gain is approximately flat to 32 kHz, and then there is a sharp decrease above this frequency, in accordance with the simple model. The slope of the sharp falloff appears such that the gain takes slightly less than one octave to reach zero. It should be kept in mind, however, that more precise measurements of this effect are needed because: (1) measurements have been made at only 8-kHz intervals, (2) gain determinations in regions where the gains are small are the least accurate, because of the poorer signal-to-noise ratios.

The development of responses to high frequencies presented in Figs. 7–9 is entirely consistent with the view that the major developmental changes are due to a progressive increase in the base cutoff frequency with age. At the earliest age (14 dab) the base cutoff frequency appears to be only about 8 kHz. That is, the passive threshold increases sharply above 4 kHz, while the cochlear amplifier gain is quite low for $f_2 = 16$ kHz and above. The gain reaches zero in slightly more than an octave above 8 kHz, at the youngest age. Only a few days later, by 18 to 23 dab, the base cutoff frequency is apparently near its adult value, which is about 32 kHz. Little change occurs from then to adulthood in the response at the high frequency edge. A shift of about two octaves in

the base cutoff frequency between 14 dab and adult is quite compatible with the shift of about 1.5 oct found for characteristic frequencies in the basal turn of the cochlea during the same period (Arjmand *et al.*, 1988).

It is important to note that the finite base cutoff apparently causes a sharp increase in passive thresholds, e.g., the steepness of the decline in passive sensitivity at high frequencies for 14–16 dab in Fig. 7. If we consider frequencies for which the active gain is still quite small, the stimulus level to reach nonlinear levels can be quite high. For example, at 32 kHz and 16 dab, this stimulus level, L_x, is over 100 dB SPL. This is a demonstration of the fact that the stimulus levels which evoke essentially active emissions will almost certainly be at relatively low levels at the active place *in the cochlea*, but will certainly not seem like low stimulus levels *in the ear canal* when the passive transmission path has such a high threshold.

Another way to put this is to say that in order to study the development of hearing, it is sometimes necessary to use very high stimulus levels, especially at high frequencies, to enable adequate signal levels to reach areas in the cochlea sufficient to fully activate the cochlear amplifier. High stimulus levels in the ear canal required to elicit a response do not necessarily imply high levels in the cochlea.

2. Developmental changes in cochlear amplifier gain

At the lowest frequencies studied, the cochlear amplifier is apparently nonfunctional at the onset of hearing (note lack of response at $f_2=1$ kHz for 14-dab animals in Fig. 7). The cochlear amplifier gain at 1 kHz increases slowly, reaching adult values only at 23 dab, when the EP also has reached adult values (Fig. 8). In contrast, the cochlear amplifier gain is already at adult levels at 14 dab for the midfrequencies ($f_2=4$ to 8 kHz), at an age when the EP is only about 25 mV. The responses at higher frequencies, as we have noted above, are consistent with the view that the elements of the cochlear amplifier are fully functional in the basal turn of the cochlea, but that the amplifier's response is limited by the development of the base cutoff frequency.

Unlike the response at the mid frequencies, the cochlear amplifier in the apex appears to require the full adult EP to attain adult gain values (Fig. 8). Immaturity of one or more of the cochlear amplifier elements (e.g., outer hair cells) in the cochlear apex at the early ages may contribute to the lack of response, of course. This is consistent with anatomical evidence of delayed development in the cochlear apex (Rubel, 1978).

During the period 14 to 18 dab, when the EP rises sharply, the cochlear amplifier gain also increases sharply at all frequencies (Fig. 8). This seems to confirm the view that the high mammalian EP is the ''battery'' for the cochlear amplifier (Ashmore, 1987; Neely and Kim, 1986).

For mid and high frequencies, however, it seems an important and surprising finding that the cochlear amplifier gain actually *decreases* subsequently during development, during the 11 day period from 18 to 29 dab. While a general decrease in gain from 23 dab to adulthood was noted earlier (Mills *et al.*, 1994), results presented here imply a remarkably uniform decrease in gain from 23 to 29 dab, for $f_2=8$ to 32 kHz (upper panel, Fig. 8). During this entire period, the EP is presumably at adult levels and quite stable.

It is interesting to speculate that this reduction in gain may be connected to another surprising finding, the recovery of cochlear function with a subnormal EP that was shown to occur in adult gerbils at middle frequencies ($f_2=8$ kHz; Mills *et al.*, 1993). This is believed to be due to an adaptation mechanism, with a time constant of about 15 min. For any adaptation mechanism to function successfully under normal conditions, of course, there must be reserve power in the system. That is, for the cochlear amplifier gain to recover its normal value with a subnormal EP, it must have power in reserve at the normal EP. The existence of the adaptation mechanism suggests that the reduction in gain observed with development is not due to a change in cochlear mechanics from 18 to 29 dab which limits the gain available. Rather, the set point of the cochlear amplifier at higher frequencies may be being reduced to more ''desirable'' levels. This conclusion is supported by the fact that the passive threshold measures, L_x and L_p, at midfrequencies changed very little over this development period.

Why would the maximum gain possible at each frequency not be the most desirable gain for the cochlear amplifier? Or conversely, why provide more gain than is the most desirable? Considering a simple analogy indicates some answers. The cruise control in an automobile is an example of a feedback mechanism with a set point. Obviously, a cruise control will not work without excess power. Excess power is generally needed in an automobile to climb hills, to achieve higher than normal speeds, but obviously it is not a good idea to drive at the maximum power available at all times.

Reserve power in the cochlear amplifier is likely to be needed for similar reasons. From the measurements here (Fig. 8) it seems quite possible that the amplifier may need to have excess gain available at 8 kHz so that sufficient gain is present at both lower and higher frequencies. While it might seem that the EP could instead be varied along the cochlear duct, the large differences in EP required to equilibrate the gain over relatively short intervals may not be possible. It is known that the scala media is normally nearly isopotential, even in the developing animal (McGuirt *et al.*, 1995). In addition, it is probably useful to have reserve gain, when this can be easily provided (i.e., by a constant, high EP), to compensate for declines in outer hair cell function or EP, as the organism suffers loss from noise damage or aging.

Why not operate at the maximum gain available? Obviously, it would be more difficult to detect sounds at, say, 25 kHz if all sounds near 8 kHz were amplified much more strongly. Further, a very high gain may lead to oscillations in a feedback amplifier system (i.e., spontaneous emissions). Finally, a vulnerable system with high gain is likely to be more easily damaged (Kanno *et al.*, 1993).

The evidence in Fig. 8 is that, once the EP has stabilized (by about 22 dab) the set point for the cochlear amplifier is adjusted downward over the next 6 days. It cannot be assumed that the intrinsic time scale for changing the set point is necessarily as high as 6 days, however. The passive thresholds are decreasing during this same time (Fig. 9) and the

combined threshold is remarkably constant (Fig. 11). The actual set point may therefore be closely following a ''desired'' value, to keep the hearing threshold approximately constant. The variations observed (Fig. 7) even suggest that the adult cochlear amplifier may compensate for a minimum in the passive threshold near 4 kHz by creating a minimum in the gain in that frequency range.

3. Comparison with other measures of hearing function

In Fig. 11, we compare the derived combined threshold measure ($L_x - A_c$) for our 42–46 dab group with the behavioral data by Ryan (1976), shifted vertically for comparison. The agreement seems quite good for the slopes of the high-frequency thresholds, and allows for an interpretation of the change in slope which was originally noted by Ryan. From 16 to about 32 kHz, the moderate slope can be attributed to an increase in passive threshold, but a relatively constant cochlear amplifier gain (Fig. 7). From 32 kHz on, the passive threshold changes relatively little, while the cochlear amplifier gain drops very rapidly, causing a steeper slope. In contrast to the excellent agreement with the high frequency slopes, the discrepancy in slope at the lowest frequencies (1 kHz) between our combined threshold estimate and Ryan's data is significant, and the reason for the discrepancy is not known.

There are no similar behavioral measures of hearing function for younger gerbils. There are measurements of cochlear microphonic responses measured at the round window, and neural thresholds in the ventral cochlear nucleus (Woolf and Ryan, 1984, 1985, 1988). Neural thresholds are displayed in Fig. 11(b) for comparison with our derived thresholds for young gerbils. Overall, the same general trends are seen in both studies: There is a greater improvement in thresholds at higher frequencies than lower, with the greatest improvement occurring in the range 16 to 32 kHz. Further, the threshold *slopes* at both low and high frequencies are quite similar for the two measures. Finally, there is more agreement than may be obvious, when one takes into account the fact that the neural thresholds were obtained with open bulla. This has the effect of artificially lowering the thresholds at frequencies below 4 kHz in the 14-dab neonates by about 10 to 15 dB (Cohen, 1993; Mills *et al.*, 1994).

The major difference between the two measures is the much larger overall improvement in threshold for the neural response that occurs from 14 to 16 dab. It is likely that this improvement can be attributed to the enhancement of *inner* hair cell function by the increase of EP during this period (Sewell, 1984c; Rübsamen *et al.*, 1995).

In comparing our results to other measures, it is important to realize that our results are obtained from emission measurements which are intended to estimate cochlear mechanical responses. These results will not indicate or be affected by the maturity of neural function. Further, it is important to note that our results imply that if one were to measure properties which depend on the overall peripheral response (e.g., neural thresholds) one would get the impression that the cochlea is essentially adult-like at 23 dab, and that no important changes occur thereafter. However, this impression would not be correct. We find a significant improvement in passive thresholds during the period 23 dab to adult, which is generally countered by a downward adjustment of the gain of the cochlear amplifier. This adjustment appears not only to take place in general terms, but on a frequency specific basis. For example, the passive threshold improvement is smallest at the lowest frequencies (Fig. 9) and the decrease in the gain of the cochlear amplifier is also lower there (Fig. 8). The ability of the cochlear amplifier to change its set point on a frequency specific basis in order to compensate for improvements in the passive conductance properties seems an intriguing result of this study.

IV. CONCLUSIONS

(1) At the earliest age that can be studied with emissions (14 dab), the cochlear amplifier at middle frequencies (4–8 kHz) is fully functional and the gain is at adult levels (Fig. 7). The gain reaches adult levels at 16 kHz by 16 dab. There is a place code shift of about 1.5 octave in the basal turn of the cochlear from 14 dab to adulthood (Arjmand *et al.*, 1988). The development of the cochlear amplifier therefore cannot be responsible for the place code shift at these frequencies; the shift must be due primarily to passive changes in basilar membrane mechanics. These passive changes appear to be related to a developmental shift in the passive base cutoff frequency, which increases about two octaves from 14 dab to adult.

(2) At midfrequencies, both active and passive mechanisms contribute to hearing function at 14 dab. The high auditory thresholds at this age appear primarily due to high passive thresholds, which are partly due to effects of a lower base cutoff frequency in the youngest animals, and partly due to a uniform conductive immaturity in passive input transmission.

(3) A normal EP is not required for adequate cochlear amplifier operation at midfrequencies (Fig. 8). However, it appears to be required to obtain full function at the upper and lower frequency extremes, since these do not reach adult function until the EP is near adult levels. This seems particularly evident for frequencies near 1 kHz. Therefore, the ''excess power'' available at 8 kHz may, at least in part, exist in order that there is the minimum power needed for optimal functioning at the extremes in frequency. It may also be useful to provide reserve power at mid frequencies to compensate for noise damage or aging.

(4) The set point for the gain of the cochlear amplifier appears to change in a frequency-specific manner with age (Fig. 8). For example, the gain at 8 kHz subsequently decreases 12 dB after reaching its maximum at 18 dab. This change appears to be driven by the passive response; that is, the gain set point compensates for the improvement in passive response, decreasing as the passive response increases. The time scale is about 6 days, but the minimum time needed by the adjustment processes is not known. The mechanisms for changing the set point are completely unknown. The result of the gain control is an adult auditory threshold function which is relatively flat across frequency from 2 to 32 kHz (Fig. 11).

ACKNOWLEDGMENTS

We would like to thank Brandon Warren for extensive assistance with software and hardware development on this project, Doug Keefe for helpful discussions regarding high frequency calibration techniques, and Ann Brown, Bill Lippe, Susan Norton, Jude Steinberg and three anonymous reviewers for useful comments on earlier versions of the manuscript. Support was provided by research grants DC 00116-01 and DC 00395 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

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