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Development of otoacoustic emissions in gerbil: Evidence for micromechanical changes underlying development of the place code *

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The development of the acoustic distortion product (ADP) $2f_1 - f_2$ was studied in gerbils, beginning 12 days after birth (P12). ADPs were measured as a function of stimulus frequency region (1.0 to 13.0 kHz) and level (10 to 80 dB SPL). There was an orderly progression in the appearance and maturation of the emissions, with responses to high-frequency stimuli ($f_2 = 13.0$ kHz) appearing first, at P13–14. Responses to mid and high frequencies ($f_2 = 3.9$ to 13.0 kHz) matured earlier than responses to lower frequencies. Responses to low-frequency stimuli ($f_2 = 1.3$ KHz) did not appear until P18–19 and were not mature until after one month of age. The first emissions to develop in a given frequency region had elevated thresholds, were reduced in amplitude, and displayed monotonic input-output functions. As the auditory system matured, emission growth functions became non-monotonic displaying saturation, but initially retained a reduced dynamic range. Data from the developing gerbil suggest that initially its cochlear mechanics are passive and that active elements associated with normal outer hair cell function mature first in the basal turn and last near the apex. Furthermore, the development of active nonlinear elements underlying ADP generation is consistent with the development of frequency selectivity and developmental shifts in the place code which have been demonstrated in the gerbil.

Acoustic distortion products; Auditory development; Place code; Cochlear mechanics; Gerbil

Introduction

The early stages of development of many physiological and behavioral responses to tonal stimuli are dominated by responses to low-frequency sounds. However, anatomically the basal portions of the cochlea, known to respond best to higher frequencies in adults, mature first. Rubel (1978) proposed that this paradox may be due to a shift in the place code in the developing cochlea, with the basal end initially responding best to frequencies 0.5 to 1.5 octaves lower than in adults of the same species.

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Place code shifts

Initially, this hypothesis was tested and supported in the avian ear. As predicted, the place of maximum damage produced by a given high-intensity pure tone shifts apically along the basilar papilla as a function of age during the final stages of hearing development (Rubel and Ryals, 1983; Ryals and Rubel, 1985). In addition, within the chick brainstem nuclei, the best frequency of neurons shifts by 1.5 octaves between embryonic day 16 (E16) and 3–4 weeks posthatching (chicks begin to hear at E12–13) (Lippe and Rubel, 1983, 1985; Lippe, 1987). This theory is not without its detractors. Cotanche et al. (1987) suggest that developmental changes in the location of pure-tone induced damage can be accounted for by developmental changes in hearing sensitivity and thus, suspectibility to noise-induced trauma. Careful inspection of their summary plots, however, reveals a shift even when corrections are made for sensi-

tivity changes. In addition, Manley et al. (1987) failed to find postnatal changes in the characteristic frequency of ganglion cells projecting to tall hair cells. These authors compared the characteristic frequency of low frequency (< 1.5 kHz) ganglion cells in chicks at postnatal day 1 (P1) and P8. Again, careful inspection reveals that their data are consistent with those of Lippe and Rubel (1983, 1985) and Lippe (1987) who report no *postnatal* changes in place representation for low frequency (< 1.5 kHz) regions of the brainstem auditory nuclei, and with Ryals and Rubel (1985) who reported that the position of damage of tall hair cells appeared insensitive to either age or frequency.

Recent work in the gerbil is generally consistent with the hypothesis of an ontogenetic shift in the place code. In the first turn of the gerbil cochlea, cochlear microphonic (CM) cut-off frequency shifts to progressively higher frequencies as a function of age (Harris and Dallos, 1984; Arjmand et al., 1988). In addition, direct recordings from basal ganglion cells demonstrate a shift in characteristic frequencies (Echteler et al., 1989). However, in the second turn, no changes in CM cut-off frequency were observed by Arjmand et al. (1988) at the ages tested. Single-unit studies in the gerbil lateral superior olive (LSO) also indicate that the characteristic frequency of units in a given location increase as a function of age (Sanes and Rubel, 1988; Sanes et al., 1989). In these studies, units receiving input from both upper-middle and basal turns appeared to show developmental increases in their characteristic frequency. However, in low-frequency regions (< 2.5 kHz) no changes were seen over the period studied (P14 to adult). These data are consistent with those discussed above and with the 2-deoxyglucose developmental mapping results reported by Ryan and Woolf (1988).

Several explanations of ontogenetic changes in the place code have been offered, including changes in the middle ear, changes in the mass and stiffness of the basilar membrane, maturation of outer hair cells, or changes in intrinsic tuning of hair cells (Zakon, 1986). In all likelihood many factors contribute to the observed changes, but recent work suggests that changes in cochlear mechanics may play a fundamental role.

Active cochlear mechanics and otoacoustic emissions

For several years, theoreticians and experimentalists alike were perplexed by the apparent discrepancy between the broad tuning and relative insensitivity of the basilar membrane and the sharp tuning and high sensitivity of single 8th nerve fibers. This apparent discrepancy lead Evans and Wilson (1973) to propose a 'second filter' to provide sharpening between basilar membrane motion and the 8th nerve response. However, subsequent measurements suggested that basilar membrane motion in the healthy cochlea with intact outer hair cells is as sharply tuned as 8th nerve fibers (Rhode, 1978; Khanna and Leonard, 1982; Sellick et al., 1982). In addition, Rhode's data (1978) indicated that basilar membrane motion increases nonlinearly with intensity. Thus, current models of cochlear mechanics must account for the high sensitivity and sharp tuning of the cochlear partition.

The most successful models of cochlear mechanics (i.e., those which accurately mimic physiological data) incorporate highly nonlinear, physiologically vulnerable, active biomechanical elements or mechanisms (e.g. Kim, 1986; Neely and Kim, 1983, 1986). Functionally these elements are termed the cochlear amplifier, and anatomically they most likely involve the outer hair cells (OHCs) operating in conjunction with the basilar and tectorial membranes. Feedback between OHCs and the basilar membrane acts to improve sensitivity and frequency selectively in a narrow region of the basilar membrane (Neely and Kim, 1983, 1986). Whether the 'active processes' are energy injecting or merely energy enhancing from an engineering viewpoint is the subject of signicant debate. However, whatever the exact mechanisms underlying the cochlear amplifier it is metabolically vulnerable. Absence of active elements (i.e. standard passive models and damaged or missing OHCs) results in elevated thresholds and broad tuning, with the characteristic frequency of a given cochlear place shifted downward by one-half to one octave (Neely and Kim, 1986).

Evoked otoacoustic emissions (EOAEs), including those evoked by clicks, tone pips, continuous tones, and two tones at distortion product frequencies, are a primary manifestation of active biomechanical processes within the normal cochlea

(Kemp, 1978, 1986, 1988). In response to external stimulation, non-linear gain is introduced in the amplifier, resulting in oscillations which propagate out of the cochlea to the ear canal in the form of otoacoustic emissions (OAEs). OAEs increase nonlinearly with stimulus level, are frequency specific * and are highly vulnerable to cochlear insults such as noise, ototoxic drugs, hypoxia and death. The presence of EOAEs in response to low- to moderate-level stimuli is associated with normal OHC function at the place appropriate to the stimulus frequency (Kemp, 1986, 1988). Thus, EOAEs are a sensitive non-invasive tool for studying active processes involved in cochlear mechanics in a place- and frequency-specific manner in vivo. In the experiments reported here we have examined the development of acoustic distortion product emissions to low- and moderate-level stimuli. We refer to these as representing the contribution of an 'active process' because in the adult they meet the criteria noted above. Other 'active mechanisms' which may contribute to responses at stimulus levels above 65–75 dB SPL have not been considered in our observations or our preliminary developmental models.

Adult gerbils have particularly strong acoustic distortion products (e.g. Kemp and Brown, 1984; Brown, 1987). That is, in response to two tones, f_1 and f_2, of appropriate frequency ($f_1 < f_2$; $1.0 < f_2/f_1 < 1.5$) and amplitude, the cochlea generates nonlinear distortion products, the most prominent of which is at $2f_1—f_2$, the lower cubic difference tone or CDT. CDTs can be measured in 8th nerve responses as well as in the ear canal (Kim et. al., 1980). They are dependent upon cochlear status in a place specific manner (Lonsbury-Martin et al., 1987; Martin et al., 1987). The observation that the neural responses of developing gerbils (Echteler et. al., 1989; Sanes and Rubel, 1988; Sanes et. al., 1989; Yancey and Dallos, 1985) are much like those of adult gerbils and other mammals with damaged outer hair cells (e.g., Evans, 1974; Schmiedt, 1981; Liberman, 1984; Liberman and Dodds, 1984, 1987; Brown et al., 1989), suggested to us that the development of the ADP $2f_1—f_2$ in gerbil could provide insight into the hypothesis that changes in cochlear micromechanics underlie ontogenetic changes in the place code and frequency selectivity. Specifically, we hypothesized that the development of active mechanisms associated primarily with the development of the outer hair cells underlies the one-half to one octave ontogenetic place code shifts observed in the gerbil. Furthermore, we hypothesized that these changes would be reflected in ontogenetic changes in acoustic distortion products produced by the gerbil cochlea.

* Transient evoked emissions are 'frequency specific' in that, in the absence of a spontaneous emission, evoked otoacoustic emissions only contain energy at frequencies represented in the stimulus. In the case of click evoked emissions, multiple emission generators are simultaneously stimulated. Weaker components may be cancelled, resulting in an absence of energy at particular frequencies, but energy will only be present at frequencies contained in the stimulus.

Methods

Animal preparation

Seventy five gerbils (*Meriones unguiculates*) ranging in age from postnatal day (P) 13 to 102 were studied. Young animals were either 13–14 ($N = 13$), 15–16 ($N = 12$), 17 ($N = 5$), 18–19 ($N = 15$), 20–22 ($N = 8$) or 28–30 ($N = 4$) days old. Adult animals ($N = 18$) ranged from 60 to 102 days. Gerbils used in this study were obtained from a commercial supplier (Tumblebrook Farms, Brookfield, MA) or born in the laboratory gerbil colony from stock obtained from this supplier. Litters born in our colony were culled to six animals. All animals had free access to food and water, and were maintained in the University of Washington vivarium facilities meeting AAALAC standards.

Animals were given a single injection of atropine sulfate (0.05–0.1 mg, i.m.), followed by a pre-anesthetic tranquilizer, ketamine hydrochloride (20 mg/kg, i.m.), then anesthetized with sodium pentobarital (40 mg/kg, i.p.) or chloral hydrate (400 mg/kg, i.p.). Young animals always received chloral hydrate. Supplemental doses of both the tranquilizer and the anesthetic were administered as needed during the experiment to eliminate nociceptive responses.

Using an operating microscope, the external ear canal was checked for debris and the tympanic membrane was inspected to insure it was intact

and clear. The pinna and outer third of the external ear canal were surgically removed.

Animals were secured to a custom-designed head holder using cyanoacrylic glue, and placed in a double-walled IAC booth. Rectal temperature was maintained at 37 degrees Centigrade by an automatically regulated heating pad placed underneath the animal during the experiment.

Stimulus generation and response measurements

Two sinusoidal stimuli, f_1 and f_2, $f_1 < f_2$, were presented simultaneously through separate channels. Stimuli were generated by a Krohn-Hite 5910B and a Wavetek 134 or 171 Arbitrary Function generators, then attenuated by separate custom-designed digital attenuators (RD Systems). Two modified ER-2 earphones were connected to separate sound delivery tubes of an Etymotic ER-10B low-noise microphone probe assembly. The probe tip was modified to fit snugly into the external canal. The sound pressure level outputted by each earphone at the probe tip was calibrated from 0.2 to 15.0 kHz using the ER-10B microphone and a Hewlett Packard 3561A Signal Analyzer. * This information was transferred to a PDP 11/73 computer for use in automated stimulus control. Calibrations were performed at the beginning of each session, and repeated any time the animal exhibited significant movement or there were significant changes in responses during a session.

Ear canal sound pressure was measured using the ER-10B low-noise microphone, amplified 40 dB (Ithaco 144L Low-Noise Preamplifier) and fed to the signal analyzer. Eight spectra were sampled and averaged for each stimulus condition. For ADP measurements, the signal analyzer was centered at $2f_1 - f_2$ with a span of 0.2 kHz, yielding a frequency resolution of 0.25 Hz.

f_2 was either 1.3, 1.82, 2.6, 3.9, 5.2, 9.75 or 13.0 kHz and the f_2/f_1 ratio was always 1.3. Stimulus levels, L1 and L2, were always equal and ranged from 10 to 80 dB SPL in five dB steps. The sound pressure level and frequency of each stimulus in the ear canal were checked and adjusted for each presentation. Significant acoustic distortion was generated in a test cavity, as well as in the ear canals of gerbils more than 24 hours post-mortem, for stimulus levels 85 dB SPL and above. Therefore, the maximum stimulus level was set at 80 dB SPL. The maximum possible acoustic artifact in the gerbil ear canal is represented by the responses obtained 24 or more hours post-mortem (see Fig. 3).

The order of frequencies was randomized across animals, but each series always began at the lowest stimulus level in order to minimize the effects of adaptation and fatigue. After all frequencies were tested in an animal, the first frequency examined was reevaluated to insure stability of the preparation. If differences of more than 5 dB occurred in threshold or ADP level at a given stimulus level, the source of the shift was investigated, and the series repeated. If stable results could not be obtained, the animal's data were discarded.

A subset of animals were reexamined at various intervals ranging from 3 min to 24 h following death due to a lethal intracardiac injection of sodium pentobarbital. Rectal temperature was maintained at 37 degrees Centigrade until each experiment was completed. The stimulus paradigm was the same as that used with live animals.

Results

Thresholds

Acoustic distortion product threshold was defined as the lowest stimulus level at which a repeatable peak at least 2.0 dB above the noise floor occurred in the spectrum of the ear canal sound

* As one anonymous reviewer of a previous version of this paper noted, there are inherent difficulties in the calibration of frequencies above 7.0 kHz, especially in closed cavities. That is, standing waves with one or more nodes may occur within 1 or 2 mm from the tympanic membrane when the frequency is 14 kHz in a closed cavity (Stinson, 1986). The reviewer suggested that specifying x dB SPL in an occluded ear canal at one point located a certain distance from the tympanic membrane may be inadequate. While we are aware of this problem and the fact that calibration differences may have contributed to the larger confidence intervals in adults at $f_2 = 13.0$ kHz, it is not possible to re-calibrate post-hoc. It is worth noting that the standard deviations in ADP measurements are not significantly different at high and low frequencies. In future studies it may be desireable to follow the reviewer's suggestion and document the variability at high frequencies by repeated calibration in the same ear.

pressure at the frequency corresponding to $2f_1 - f_2$. Thus, ADP threshold is a visual detection threshold. The mean noise floor across all age groups and frequencies was −12.7 dB SPL (SD = 3.0), and ranged from −7.2 dB SPL for an ADP of 0.7 kHz (f_2 = 1.3 kHz) to −17.9 dB SPL for an ADP of 7.0 kHz (f_2 = 13.0 kHz). While the noise floor affects the detectability of emissions, there were no reliable changes in noise floor as a function of age. Furthermore, regression analyses revealed that variations in the noise floor accounts for only 26% of the variance in threshold across all age groups and frequencies. Also, although the noise floor was highest in the 0.7 kHz region (f_2 = 1.3 kHz), it accounted for only 4% of the variance in ADP thresholds in this frequency region across all age groups. Where thresholds were lowest and showed little variability, f_2 = 3.9 kHz, the noise floor accounted for 59% of the variance in emission thresholds.

Fig. 1 shows the proportion of animals in each age group which responded when f_2 was equal to a given frequency. Responses first appeared at the highest frequencies tested, f_2 = 13.0 kHz, then at the mid-frequencies, f_2 = 3.9 or 5.2 kHz and finally in the low frequencies, f_2 = 1.3 kHz. The earliest responses observed were among P13–14 animals. A few P12 animals were examined and found to have no emissions. However, consistent with reports of previous investigators (Finck et al., 1972; Ryan and Woolf, 1988), their middle ear cavities were filled with fluid and mesenchymal tissue. Ryan and Woolf (1988) reported that the gerbil middle ear is adult-like by P16, with only minor changes occurring between P14 and 16. Therefore, while middle ear characteristics probably impeded our ability to see emissions in animals younger than P13–14 and may influence the results in this group, they probably had little effect in older animals.

Among the P13–14 animals there were three distinct groups: non-responders (N = 6), who showed no emissions (at L1 and L2 ⩽ 80 dB SPL) in any frequency region examined; typical responders (N = 5), who showed ADPs in at least one frequency region examined; and atypical responders (N = 2) who showed emissions similar to P20–21 animals in the mid frequencies. All animals P15–16 and older responded to at least one frequency tested. However, not until P18–19 did all animals respond to all frequencies tested.

Mean ADP thresholds as a function of f_2

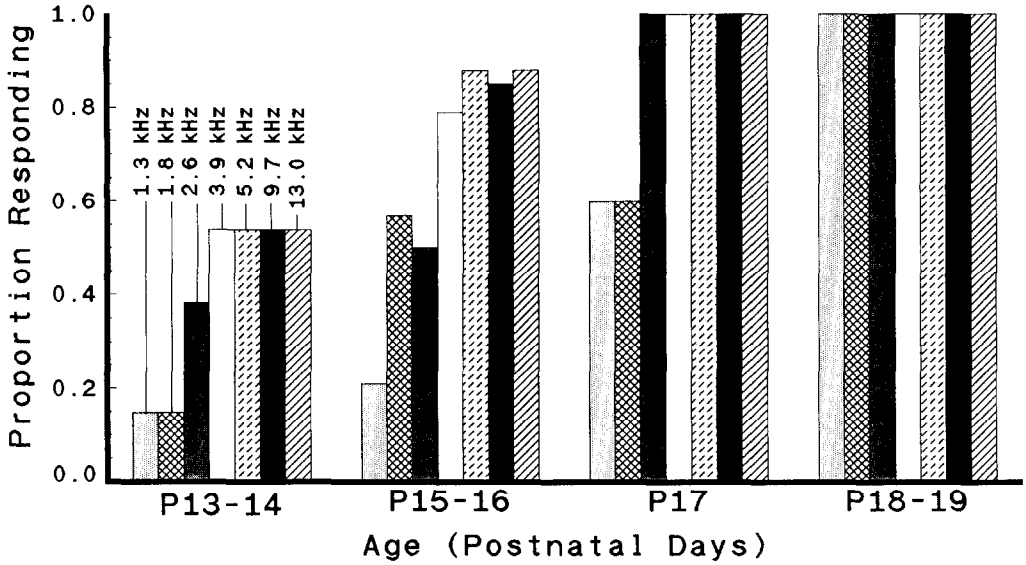

Fig. 1. Proportion of animals responding when f_2 was a given frequency region as a function of age in postnatal days. Note that for the P13–14 animals at f_2 = 1.3 and 1.82 kHz the data shown in this figure include the reponses of the two 'atypical responders' in this age group. 11 of 13 animals P13–14 did not respond at these frequencies and only data from these 'typical responders' are reported in other figures.

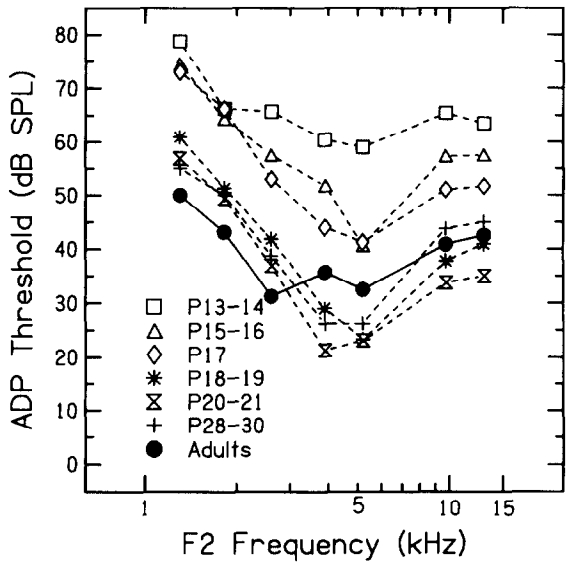

Fig. 2. Mean threshold for the $2f_1 - f_2$ ADP as a function of f_2 frequency. The parameter is age group. Only data from those animals who responded to at least one frequency examined were included in the mean. Thus, P13–14 does not include the five nonresponders in that age group.

frequency are shown in Fig. 2. The parameter is age. Only data from those animals who responded to at least one frequency at 80 dB SPL or less are included. A non-response at a particular frequency was coded as an 85 dB SPL threshold for purpose of data analysis. As seen in Fig. 2, thresholds dropped first in the mid and high frequencies. Large decreases occurred between P17 and P18–19 at all frequencies, with small improvements between P18–19 and P20–21. It is noteworthy that P18–19 through P28–30 animals had better thresholds than adults (P60–102) in the mid and high frequencies, while still showing poorer thresholds for low-frequency regions.

The shape and levels of the ADP 'audiogram' are similar to stapes driven cochlear microphonic 'audiograms' (Woolf and Ryan, 1984; Ryan and Woolf; 1988) for both developing and adult animals. There is a 5–10 dB discrepancy for stimuli in the 1.0–1.8 kHz region, emissions having higher thresholds, even in adult animals. This may reflect poorer reverse middle ear transmission of the emission or differences in the mechanisms underlying the emission and CM.

Input-Output functions: Alive vs dead

ADP levels as a function of stimulus level in five dB steps from 20 to 80 dB SPL for f_2 equal to 3.9 kHz are shown in Fig. 3. Four individual animals of different ages are shown in the separate panels. The parameter within each panel is whether the animal is alive (filled upward triangles) or dead and the time after death. Gerbil 885387, shown in the upper lefthand panel, is a P13–14 non-responder, whose responses showed no difference as a function of state. Gerbil 885378, whose data are displayed in the upper righthand panel, showed responses typical of P15–16 animals. The ADP was first observed at 45 dB SPL when the animal was alive. Emission amplitude increased as the stimulus level was increased until 60 dB SPL, saturated, and then grew sharply for 75 and 80 dB SPL stimuli. Three minutes post-mortem the growth function was monotonic. The emission threshold was elevated only 5 dB, but its amplitude as a function of stimulus level was significantly reduced until stimuli were 70 dB SPL. At 15 min post-mortem the threshold was 65 dB SPL, but responses to 70 to 80 dB SPL stimuli were unchanged. Finally, at 120 min post-mortem there were no significant responses even at high stimulus levels. Data are shown for a P28 and an adult animal in the lower left- and righthand panels, respectively. There was a very rapid decrease in emission level with a 30 dB increase in emission threshold for the P28 animal within 3 min after death. Similarly, for the adult animal there was an immediate 25 dB increase in emission threshold with a rapid drop in emission levels for stimuli below 50 dB SPL.

Similar post-mortem patterns were seen across all age groups. That is, the lower portion of the input-output function for stimuli less than 60–65 dB SPL decayed rapidly (within 3–10 min), whereas responses to higher level stimuli persisted for up to three hours after death. These results are consistent with those reported by Schmiedt and Adams (1981) and Brown (1987). We will discuss the differences in post-mortem responses as a function of age in more detail later in this paper. However, here it is important to note that the post-mortem data are strikingly similar to those obtained by Brown et al. (1989) from guinea pigs pre- and post-treatment with gentamicin, an anti-

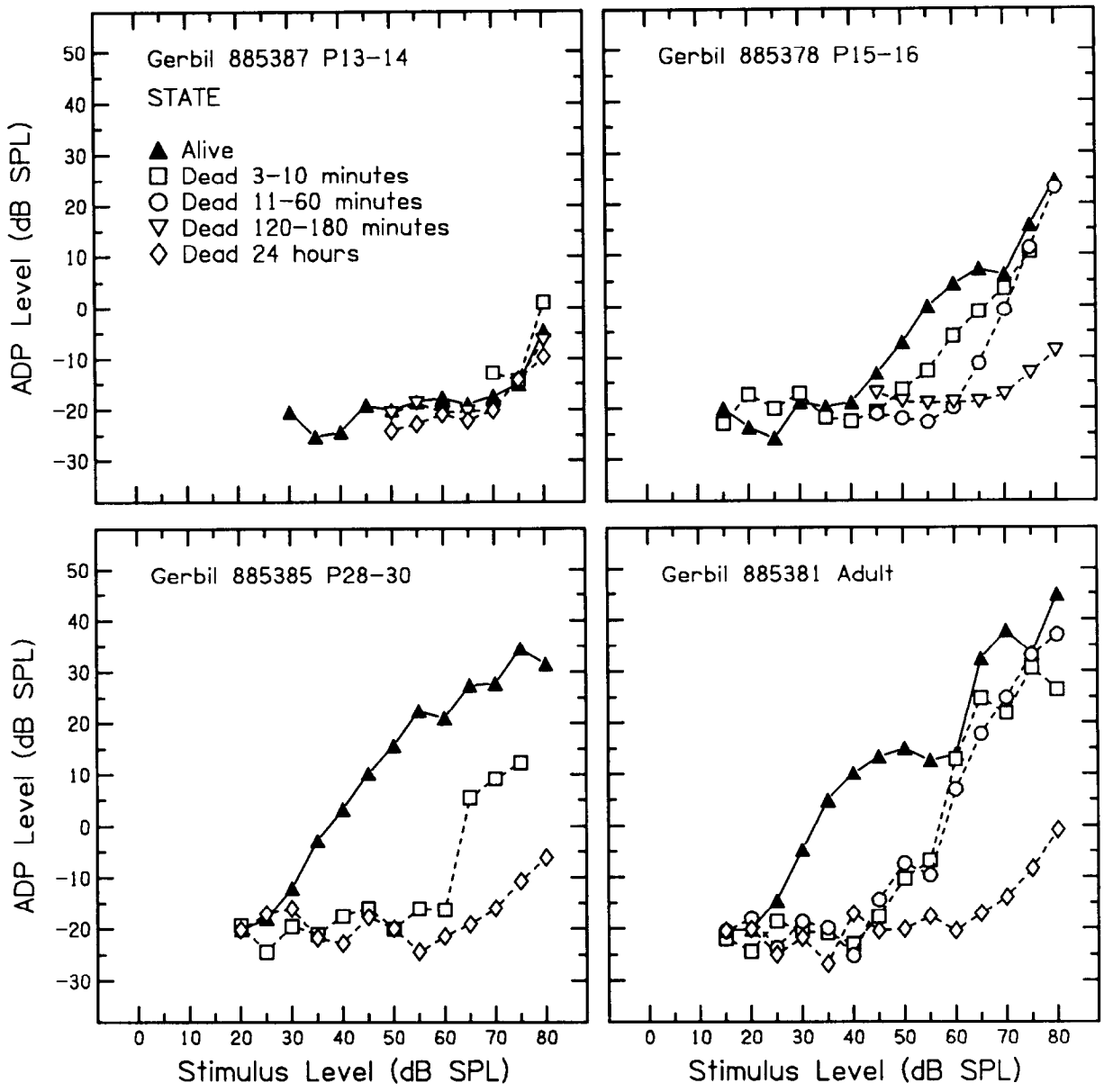

Fig. 3. ADP level as a function of stimulus level for four gerbils as a function of state, alive versus various times post-mortem. f_2 was 3.9 kHz. Gerbil 885387, shown in the upper lefthand panel is a typical P13–14 non-responder. Gerbil 885378, shown in the upper righthand panel is a typical P15–16 animal. Gerbil 885385, shown in the lower lefthand panel is a typical P28–30 animal, and gerbil 885381 is a typical adult animal.

biotic well-known for destroying outer hair cells. The hyperbolic shape of the ADP input-output functions and the post-mortem changes in shape are also nearly identical to changes in basilar membrane displacement input-output functions from alive and dead animals (Johnstone et al., 1986).

Input-Output functions: Age and frequency effects

ADP levels as a function of stimulus level in five dB steps from 10 to 80 dB SPL for a fixed f_2/f_1 ratio, 1.3, were examined across six f_2 frequencies and seven age groups. For P13–14 animals stimulus presentation began at 30 dB SPL, since responses were not seen until much

higher levels. For f_2 equal to 13.0 kHz it was not always possible to reach 80 dB SPL. Therefore, only responses to stimuli ranging between 30 to 75 dB SPL were used to statistically compare the data as a function of age and frequency. An analysis of variance and covariance (BMDP2V, UCLA) indicated statistically significant differences as a function of age for individual frequencies (df = 5, F = 30.26, $P < 0.00001$) and for frequency within an age group (df = 5, F = 66.96, $P < 0.0001$). In addition, there was a significant two-way interaction between age and frequency (df = 250, F = 2.66, $P < 0.0001$), and not surprising, a significant three-way interaction between age, stimulus level and stimulus frequency (df = 250, F = 1.97, $P < 0.00001$). The data for each age group will first be considered separately in order to concentrate on differential responses as a function of frequency. Next, trends as a function of age will be discussed.

Fig. 4 shows mean ADP level as a function of stimulus level for adult animals. The parameter is f_2 frequency. Averaging the data across animals tended to smooth the input-output functions compared to what is seen in individual animals. The data in the bottom two panels of Fig. 3 (filled triangles) are representative of adult (mature) input-output functions. Typically, the input-output

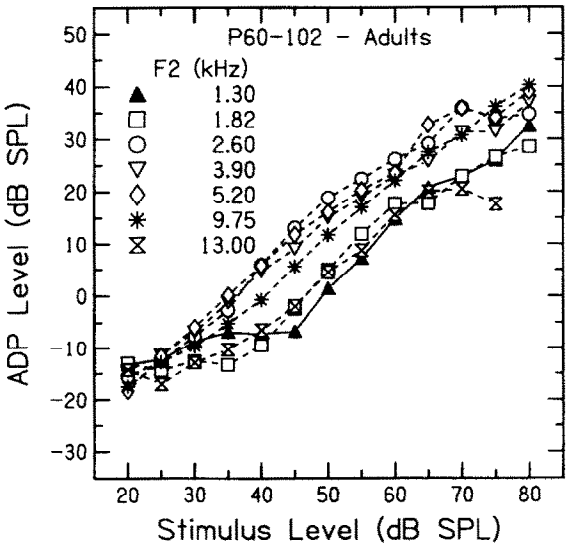

Fig. 4. Mean ADP level as a function of stimulus level for adult animals. The parameter is f_2 frequency.

functions had two distinct portions. From threshold to between 40 and 60 dB SPL, emission level increased as stimulus level was increased; then it saturated. For 75 and 80 dB SPL stimuli the emission again grew, but significantly faster than at lower stimulus levels. Often output levels decreased significantly for stimuli at or around 60 dB SPL ($\pm$ 5 dB), i.e. there was a notch in the input-output function as seen in the lower righthand panel of Fig. 3. Similar input-output functions were observed for all frequencies, although not all combinations of animals and frequencies, examined.

The highest thresholds and lowest initial amplitudes are for f_2 equal to 1.3 kHz. As can be seen in Fig. 4, emissions for f_2 = 1.3, 1.82 and 13.0 kHz display similar magnitudes for supra-threshold stimuli. Input-output functions are similar to one another for f_2s between 2.6 to 9.75 kHz. In addition, emissions for this f_2 range have higher magnitudes and lower thresholds than those for both lower and higher f_2 frequencies. Figs. 5 through 7 present data for younger animals who display quite different input-output functions across frequency. Note that in Figs. 5 through 7 the data from some intermediate frequencies are not included for clarity of presentation.

Fig. 5 shows input-output functions for the P13–14 'typical responders' in the lefthand panel and for all P15–16 animals in the righthand panel. No meaningful responses were observed for f_2 = 1.3 kHz (filled upward triangles) in either group. P13–14 animals did not respond until $f_2 = 2.6$ kHz (not shown), and then, their thresholds were elevated and emission amplitudes were low. For P13–14, the best responses, i.e. those with the lowest threshold and largest amplitude, occurred for f_2 equal to 3.9 or 5.2 kHz. Note that while there are differences in noise floor and emission threshold and amplitude in response to lower level stimuli, emission amplitudes for f_2 frequencies between 3.9 to 13.0 kHz are very similar for levels 65 dB SPL and higher at P13–14. P15–16 animals showed similar patterns as a function of frequency, with the largest responses occurring for f_2 equal to 5.2 kHz. Data for P17 and P18–19 are shown in the left- and righthand panels, respectively, of Fig. 6. It is not until P18–19 that reasonable responses are seen for f_2 equal to 1.3 and 1.82 kHz, although

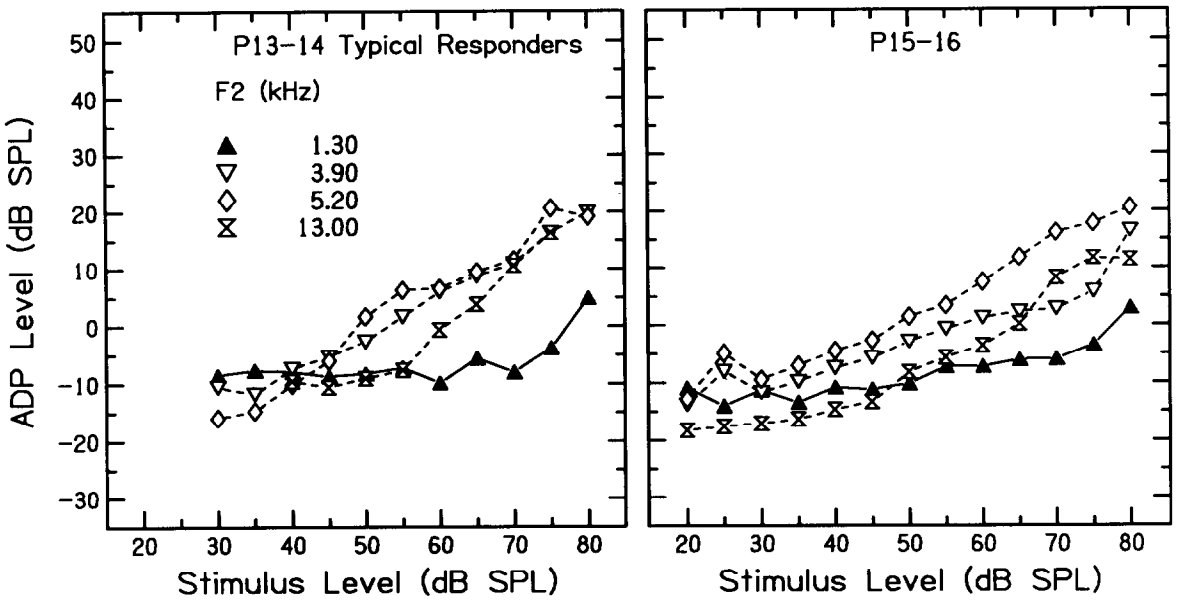

Fig. 5. ADP level as a function of stimulus level. P13–14 typical responders are shown in the lefthand panel. As in Fig. 4, the parameter is f_2 frequency. P13–14 nonresponders displayed input-output functions like those seen for $f_2 = 1.3$ kHz in this panel for all frequency regions examined. Data for P15–16 animals are shown in the righthand panel.

for other stimuli P17 animals show lower thresholds and higher amplitudes than younger animals. For P20–21 and P28–30 animals, whose mean data are shown in the left- and righthand panel, respectively, of Fig. 7, output levels have increased significantly at all frequencies. In ad-

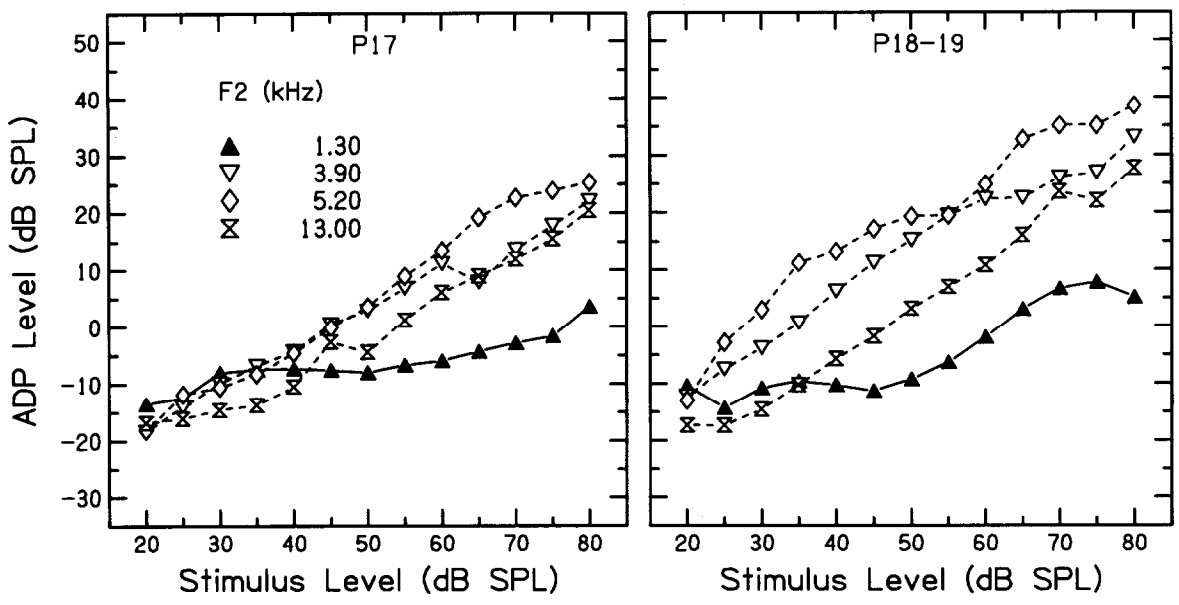

Fig. 6 Mean ADP level as a function of stimulus level for P17 animals in the lefthand panel and P18–19 animals in the right hand panel. The parameter is f_2 frequency. Symbols are the same as in Figs. 4 and 5.

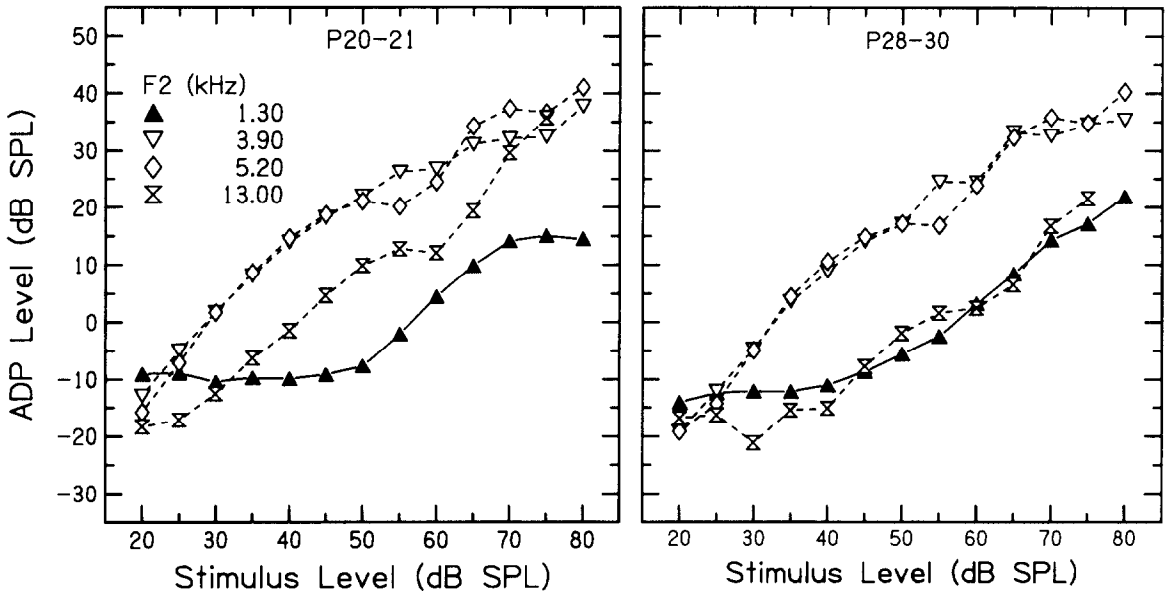

Fig. 7 Mean ADP level as a function of stimulus level for P20–21 animals in the lefthand panel and P28–30 animals in the righthand panel. The parameter is f_2 frequency. Symbols are the same as in Figs. 3, 4 and 5.

dition to thresholds decreasing and output increasing, the stimulus levels at which saturation occurs decrease.

A somewhat different perspective of the data can be gained by viewing the input-output functions at a given frequency with age as the parame-

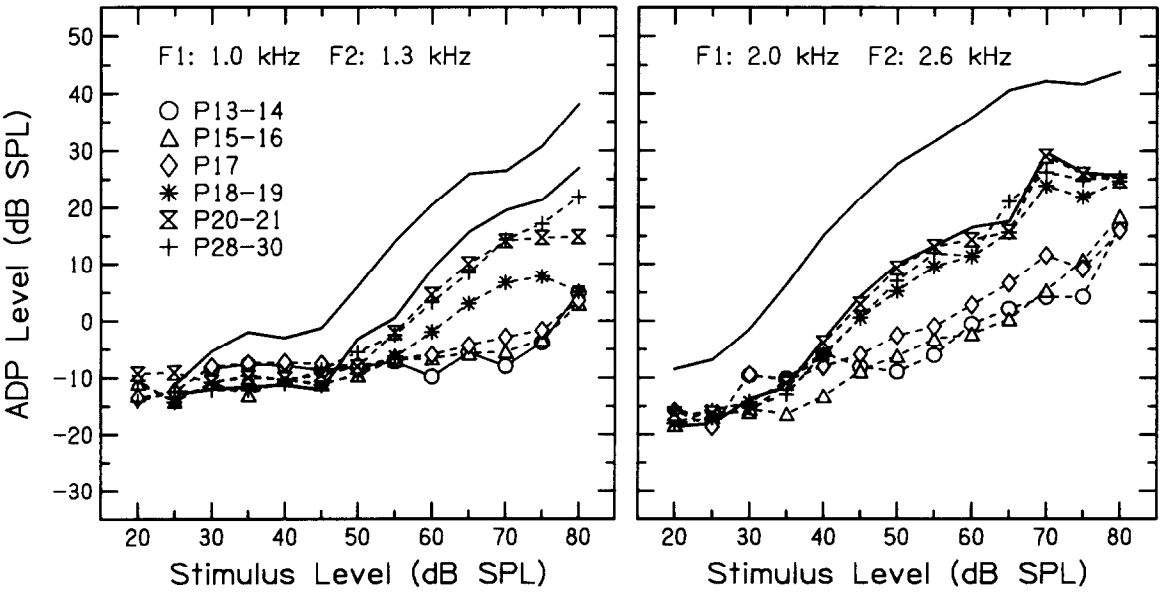

Fig. 8 Mean ADP level as a function of stimulus level for f_2 equal to 1.3 kHz in the lefthand panel and f_2 equal to 2.6 kHz in the righthand panel. The parameter within each panel is age group. The heavier solid lines without symbols indicate the 95 percent confidence limits for adult adults.

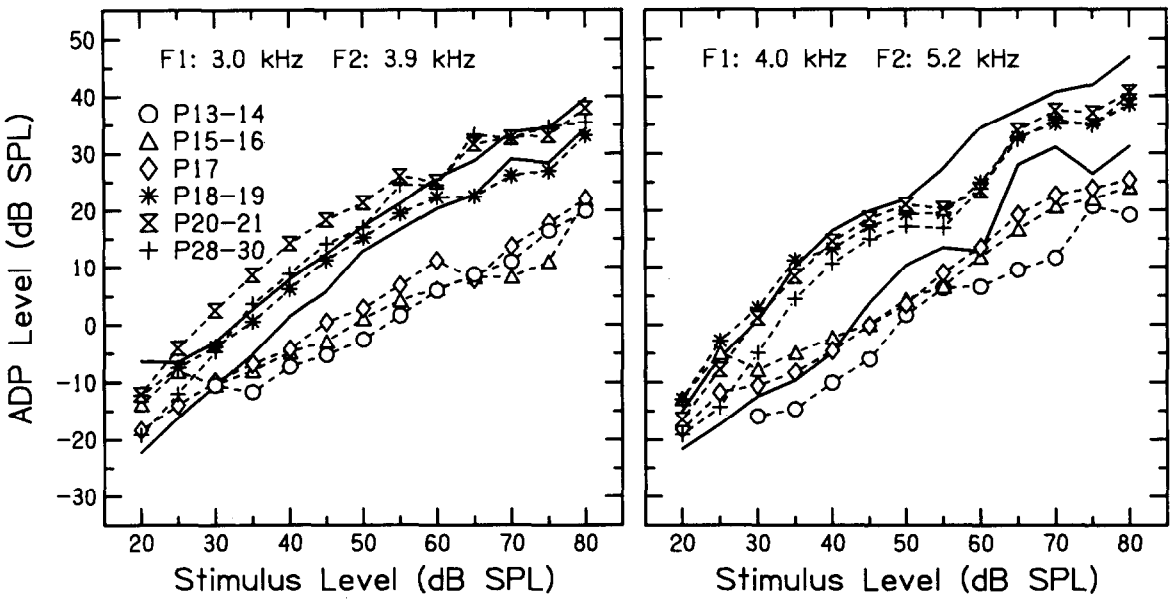

Fig. 9 Mean ADP level as a function of stimulus level for f_2 equal to 3.9 kHz in the lefthand panel and f_2 equal to 5.2 kHz in the righthand panel. Symbols for different age groups are the same as in Fig. 8.

ter. These data are shown in Figs. 8 through 10. Within each panel the solid lines without symbols indicate the 95% confidence limits for adult animals. Data for $f_1 = 1.0$ and $f_2 = 1.3$ kHz are shown in the lefthand panel of Fig. 8. No emissions are observable below 75 dB SPL for animals P17 and younger. P18–19 show low-level emissions, which first appear at 60 dB SPL, grow

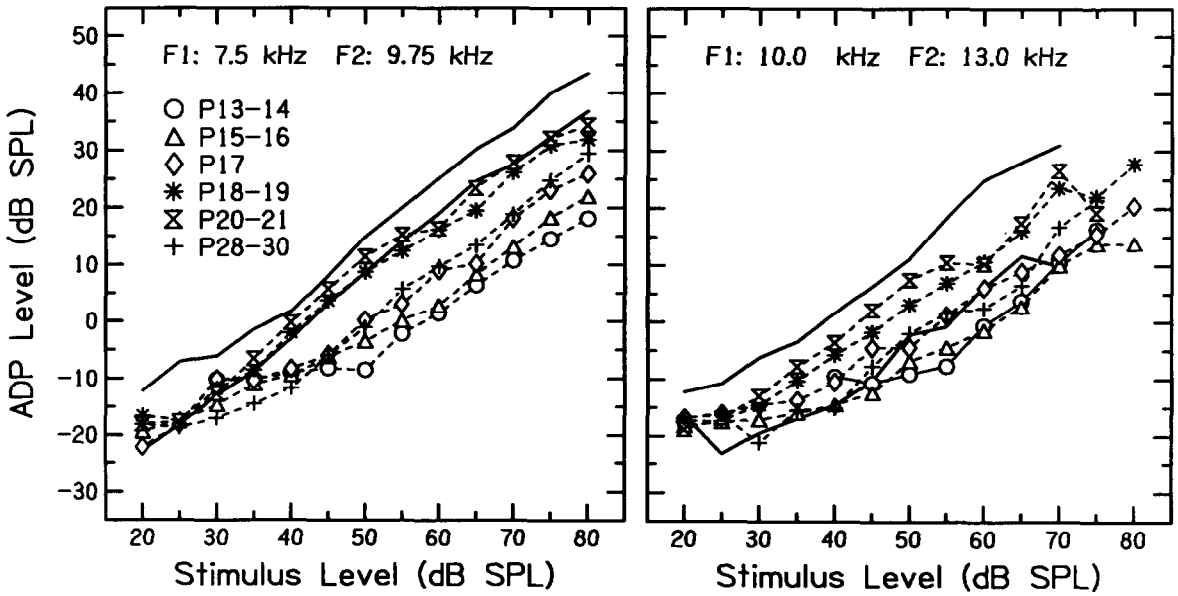

Fig. 10 Mean ADP level as a function of stimulus level for f_2 equal to 9.75 kHz in the lefthand panel and f_2 equal to 13.0 kHz in the righthand panel. Symbols for different age groups are the same as those in Fig. 8.

slowly, saturate at 75 dB SPL and decrease slightly at 80 dB SPL. At P20–21 emission levels increase for higher level stimuli with little change in threshold. This trend continues for P28–30, but responses are still outside the normal adult range. As seen in the righthand panel of Fig. 8, emissions mature more rapidly as stimulus frequency increases, but even at $f_2 = 2.6$ kHz, P28–30 animals are still slightly below the 95% confidence limits of the adult range.

As the frequencies of f_1 and f_2 are increased, responses mature earlier. In Fig. 9 the data for $f_2 = 3.9$ kHz (lefthand panel) and $f_2 = 5.2$ kHz (righthand panel) are presented. In both cases the input-output functions are within the adult range by P18–19. Fig. 10 presents data for the highest frequencies tested in the present study. At $f_2 = 9.75$ kHz and $f_2 = 13.0$ kHz the ADP input-output functions have matured to within the adult range by P18–19, and by P17 the functions for $f_2 = 13.0$ kHz are nearly mature. Thus, from the data presented in Figs. 8–10 it is apparent that ADPs evoked by low frequencies (below $f_2 = 3.9$ kHz) are markedly delayed in their maturation. Emissions to middle frequencies and the highest frequencies tested ($f_2 = 13.0$ kHz) look like those from adults by P18–19 and there is a tendency for responses to the higher frequency to mature by P17.

It is also interesting to note the age and frequency combinations at which the $2f_1—f_2$ ADP input-output functions of young animals exceed those seen in adults. At P20–21 emissions evoked by $f_2 = 3.9$ kHz (Fig. 9, lefthand panel) clearly show hypersensitivity and hyper-responsivity for low to moderate stimulus levels, whereas the emissions evoked by $f_2 = 5.2$ kHz are hypersensitive, but appear only slightly hyper-responsive to low level stimuli. Comparison of these data with the emission thresholds shown in Fig. 2 suggest some dissociation between emission input-output functions and emission thresholds. While both measures show hypersensitivity in the mid frequencies, only thresholds show hypersensitivity at the highest frequencies tested. Woolf and Ryan (1988) also observed hypersensitivity in the CM responses of young animals to middle frequency stimuli.

Discussion

Development of CDT emissions

The acoustic distortion product $2f_1—f_2$ from the gerbil cochlea develops in a consistent manner within an animal and across age groups. Responses are seen first for middle and high-frequency stimuli ($f_2 = 3.9$ to 13.0 kHz) and appear last for low frequencies ($f_2 = 1.3$ to 2.6 kHz). Mature appearing responses are found first for f_2 frequencies from 5.2 to 13.0 kHz. These results are in general agreement with those of Lenoir and Puel (1987) and Henley et al. (1989) for the rat pup although both studies used a somewhat narrower range of frequencies than the present study. Henley et al. reported data for f_2 frequencies from 6.2 to 12.5 kHz with $f_2/f_1 = 1.22$. They found that the $2f_1—f_2$ ADP matured first for the highest frequency tested, and last for the lowest frequency. Our results also are in general agreement with those reported by Lenoir and Puel (1987) for the developing rat for those frequency regions common to both studies. Lenoir and Puel examined only three frequency regions, $f_2 = 4.2$, 7.0 or 9.8 kHz. In the rat pup, $2f_1—f_2$ ADPs appeared first for $f_2 = 9.8$ kHz and last for $f_2 = 4.2$ kHz, but matured first for $f_2 = 4.2$ kHz and last for $f_2 = 9.8$ kHz. These regions roughly correspond to our mid ($f_2 = 3.9$ or 5.2 kHz) and high ($f_2 = 9.75$ or $f_2 = 13.0$ kHz) frequencies, respectively, and the sequence of appearance and maturation is similar for these regions. However, Lenoir and Puel concluded that 'OAEs clearly achieved their development from low to high frequencies'. While we would agree with this statement for the frequency regions they examined, our data indicate that when a wider range of frequencies is examined low ($f_2 = 1.3$ or 2.6 kHz) frequencies appear later and mature later than high and mid frequencies.

Our data also indicate that the development of the $2f_1—f_2$ ADP, at least in the gerbil, is consistent with anatomical changes that have been described in a variety of mammals. First, the basal and mid-basal portions of the cochlear partition develop before the apex (e.g. Retzius, 1884; Finck et al., 1972; Rubel, 1978; Romand, 1983; Lenoir et al., 1986; Whitehead, 1986), consistent with the observation that emissions occur first to high and

mid-frequency stimuli. Second, outer hair cells and tectorial membrane mature last (Pujol et al., 1980; Lenoir et al., 1986), consistent with the later development of the active, nonlinear component of ADPs. It is particularly intriguing that lateral subsurface cisternae, hypothesized to play an important role in outer hair cell motility, appear to show protracted development (Pujol et al., 1980). Woolf and Ryan (1988) reported changes in the gerbil middle ear which may have contributed to the attenuation of cochlear responses in our animals between P13 and P16. However, it is possible that small, and as yet undefined, changes in the middle ear during development could have larger effects on backward transmission than on forward transmission. However, known middle ear changes do not account for differences in emissions in older animals or across frequencies (Relkin et al., 1979). Rather changes in cochlear mechanics, particularly active cochlear processes, are most likely responsible for the observed changes in ADPs, as well as in improvements in sensitivity and frequency selectivity observed by previous investigators.

In Fig. 11 we present a phenomenological model of the development of the ADP, $2f_1-f_2$, in the gerbil. As schematically illustrated, the data suggest that developmental changes in the emission input-output functions with age are mediated by the concurrent development of two, maybe three, cochlear nonlinearities – a passive nonlinearity (Fig. 12a) associated with the basilar membrane-inner-hair-cell subsystem and one, maybe two, active nonlinearities (Fig. 12b) associated with the basilar-membrane-outer-hair-cell-tectorial membrane subsystem. Although it is biologically artificial, in Fig. 11 we have simply distinguished active and passive aspects of cochlear development by illustrating input-output functions obtained from healthy, alive animals and those obtained from the same animals post-mortem, respectively. While this is probably an oversimplification, to a first approximation, the responses of very young gerbils and those from late maturing frequency regions resemble responses seen in adult animals with known outer hair cell damage and/or post-mortem. However, there are important differences. Panel a illustrates the development of the passive nonlinearity underlying ADPs in response to

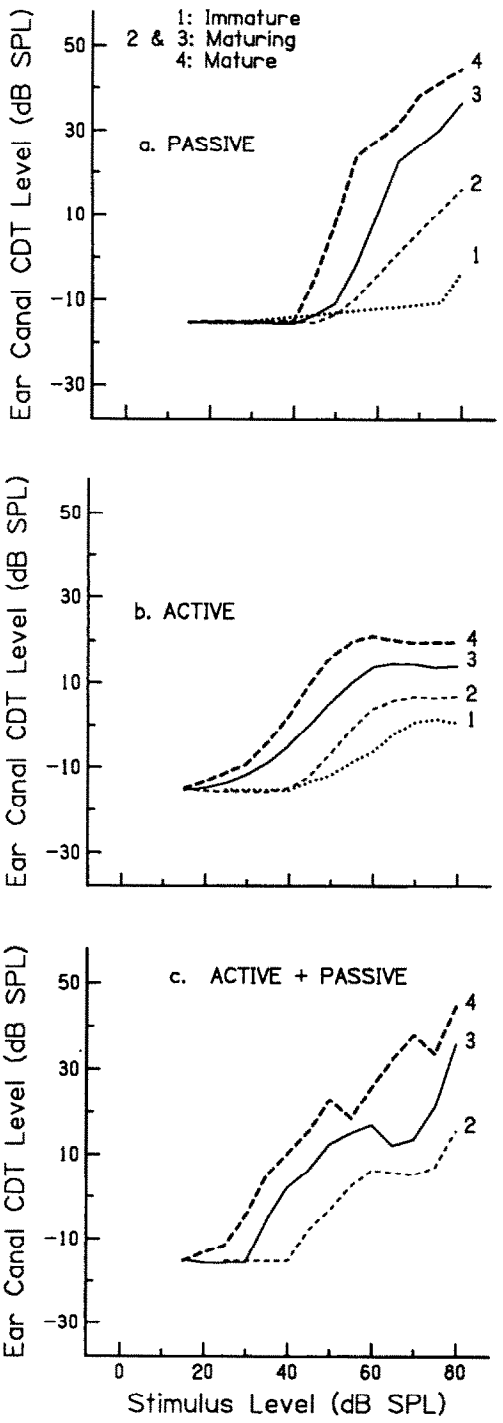

Fig. 11 Phenomenological model of the development of the active and passive nonlinearities underlying the generation of ADPs from the gerbil cochlea. See text for details.

high-level stimuli. The linear, minimal response at P13–14 and in dead animals most likely reflects limited and immature basilar membrane motion (a.1). As the basilar membrane matures, responses to high level stimuli are seen (a.2). Eventually responses to high level stimuli can grow with a slope of 1.4 to 2 (a.3–4). The slight bend in curves 3 and 4 represent residual active, nonlinearity seen for short periods of time after death in mature animals. Similar post-mortem patterns were seen in adult guinea pig basilar membrane displacement by Johnstone et al. (1986). Added to the passive nonlinearity is the development of active nonlinear elements, presumably representing the outer hair cell subsystem, illustrated in panel b. Here, it is represented as a simple saturating nonlinearity. The level at which saturation occurs was visually determined from the data in Fig. 3. As the active process matures it contributes more to the input-output functions. This is consistent with the fact that as animals get older there is a progressively more rapid and a greater decrease in emission amplitude and an increase in emission threshold immediately (within 3 min) post-mortem. This effect can be seen clearly in Fig. 3. That is, with increasing age the active component makes a larger contribution to the observed emission, particularly at low stimulus levels.

It is generally thought that outer hair cells function as biomechanical force generators amplifying basilar membrane motion through a feedback loop (Neely and Kim, 1983, 1986). The active process displays a saturating nonlinearity, such that output remains constant for inputs greater than 40 to 60 dB SPL. An important difference between healthy, developing cochleae and damaged or dead cochleae is the form of the input-output function at high stimulus levels. In dead gerbils, as seen in the present study, or in guinea pigs treated with gentamicin (Brown et al., 1989) responses to low or moderate level stimuli disappear, but responses to high level stimuli are normal - that is, the output is high with monotonic, steeply rising growth functions. In developing animals, on the other hand, the earliest input-output functions suggest weak active nonlinear mechanisms as well as weak passive components. That is, the output levels are low and the dynamic range is limited. This can be seen clearly in the changes in responses to $f_2 = 1.3$ kHz shown in Fig. 8. As the active and passive elements mature the $2f_1 - f_2$ ADP amplitude first increases at high levels and then at low levels. This suggests that active and passive elements mature simultaneously, often producing complex growth functions seen in panel c of Fig. 11.

Development of the place code

The data reported here lead us to hypothesize that two related aspects of cochlear mechanics are responsible for ontogenetic shifts in the place code. These are: 1) the development of active processes; and 2) shifts in the place of maximum displacement as the stimulus level is increased. In the remainder of this discussion we will elaborate on this model and then relate it to the literature on ontogenetic changes in place code and sensitivity to low versus high frequencies.

Proposition I

Early in development, active elements, i.e. outer hair cells appear to be non-functional. This may account for an average one-half octave downward shift in the place code in an otherwise normal cochlea.

It is well documented that there is a shift in the characteristic frequency of a particular basilar membrane location in the absence of functional outer hair cells. This shift averages $-1/2$ octave (e.g., Dallos and Harris, 1978; Robertson et al., 1980; Cody and Johnstone, 1981; Sellick et al., 1982; Liberman, 1984; Liberman and Dodds, 1984, 1987; Johnstone et al., 1986; Khanna and Leonard, 1986; Sewell, 1984). That is, for single units with normal characteristic frequencies (CFs) greater than about 1 to 2.5 kHz (depending on the species), downward shifts in the CF averaging one-half octave occur following cochlear insults involving OHC damage or dysfunction. There is some tendency for the degree of shift to increase as the normal CF increases (Liberman, 1984; Liberman and Dodds, 1984, 1987). In addition, CF thresholds are elevated, Q10s are decreased and the high frequency slopes of frequency tuning curves (FTCs) are shallower for animals with OHC damage. All of these characteristics closely resemble those seen in developing animals (e.g., Carlier, Lenoir and Pujol, 1979; Pujol et al., 1980;

Romand, 1983; Walsh et al., 1986; Puel and Uziel, 1987). The observation that the CFs of low-frequency units are not altered by OHC damage (e.g. Liberman and Dodds, 1984, 1987; Sewell, 1984) is also consistent with the observations that there are no place code shifts for lower frequencies in young animals. Lippe (Lippe and Rubel, 1983, 1985; Lippe, 1987) has reported that changes in place code for low frequencies were only seen prior to hatching whereas changes for frequencies greater than 1.5 kHz continued after hatching. Sanes and colleagues (Sanes and Rubel, 1988; Sanes et al., 1989) saw no changes in the gerbil for low frequency regions (< 1.5 and < 2.5 kHz, respectively) during normal development. These data are also consistent with Arjmand et al. (1988) who saw no change in gerbil second turn CM where the CFs averaged 2.4 to 2.6 kHz. These differences between so-called low-frequency regions and higher frequencies may represent fundamental differences in the nature of active processes in the two regions. The data from the present study support the hypothesis that active processes may play less of a role in low frequency regions. That is, in addition to being later developing, $2f_1-f_2$ ADPs for $f_2 = 1.3$ and 1.8 kHz had higher thresholds and lower output levels than emissions evoked by higher frequency stimuli even in adults. Numerous previous investigations of cochlear function and mechanics also support this hypothesis (e.g., Dallos, 1986; Johnstone et al., 1986; Patuzzi and Robertson, 1988).

Proposition II

High levels of sound produce a 'basal shift' in the young cochlea.

The second factor contributing to the observed place code shifts is that as the level of a stimulus increases to 80 dB SPL there is a significant basal shift in the place of maximum basilar membrane displacement caused by a given stimulus frequency. This shift also averages 1/2 to 1 octave as stimulus level is increased from 20 to 80 dB SPL, but may not be gradual (McFadden, 1986). Mossbauer measurements of guinea pig basilar membrane displacement as a function of stimulus level indicate a shift of about 1/5th of an octave in the place of maximum displacement when the stimulus is increased from 40 to 60 dB SPL, and another 1/2 an octave shift when it is increased from 60 to 80 dB SPL (Johnstone et al.,1986). Although there are no measurements of basilar membrane displacement in developing animals, it is parsimonious to assume that similar shifts in place of maximum displacement as a function of level occur in developing animals.

The possible effects of these two factors on the place code development are illustrated schematically for the gerbil in Fig. 12. The characteristic frequency (CF) at fixed points along the cochlear partition, i.e.. the place code, is shown. The lowest line represents the adult gerbil cochlea in terms of percent distance from the apical end. Lines a through d show the characteristic frequency as a function of percent distance from the apex (i.e. referenced to the bottom scale) for animals at various stages of maturation. Line 'd' represents normal adult gerbil place code in terms of the characteristic frequency of a particular basilar membrane location. This 'map' is derived from the central nervous system (lateral superior olive) tonotopic maps presented by Sanes et al. (1989). It assumes a logarithmic mapping of frequency on the basilar membrane and then proportional linear transforms at the central terminations. In this case the outer hair cell-basilar-membrane subsystem would be healthy and fully functional and stimulation levels would be near threshold. In c, we have depicted the place code map in the absence of functional outer hair cells. Because the outer-hair-cell subsystem increases the tuning of a particular basilar membrane place by approximately 1/2 octave (all other factors being normal) absence of the 'active processes' results in a 1/2 octave decrease in the CF of a given place. Note that we have fixed the place code for the apical 30–35% of the cochlea at this stage. This is consistent with the small changes in place code observed at lower frequencies and a relatively weak contribution of active elements to processing in the apical cochlea. In addition, the inner hair cells and basilar membrane may be abnormal or immature necessitating an additional increase in level. Between P13 and P30 these changes are probably small. Harris et al. (1986) reported that the gerbil's basilar membrane reaches adult length by P9–10, but some changes in mass occur up to P20. Unfor-

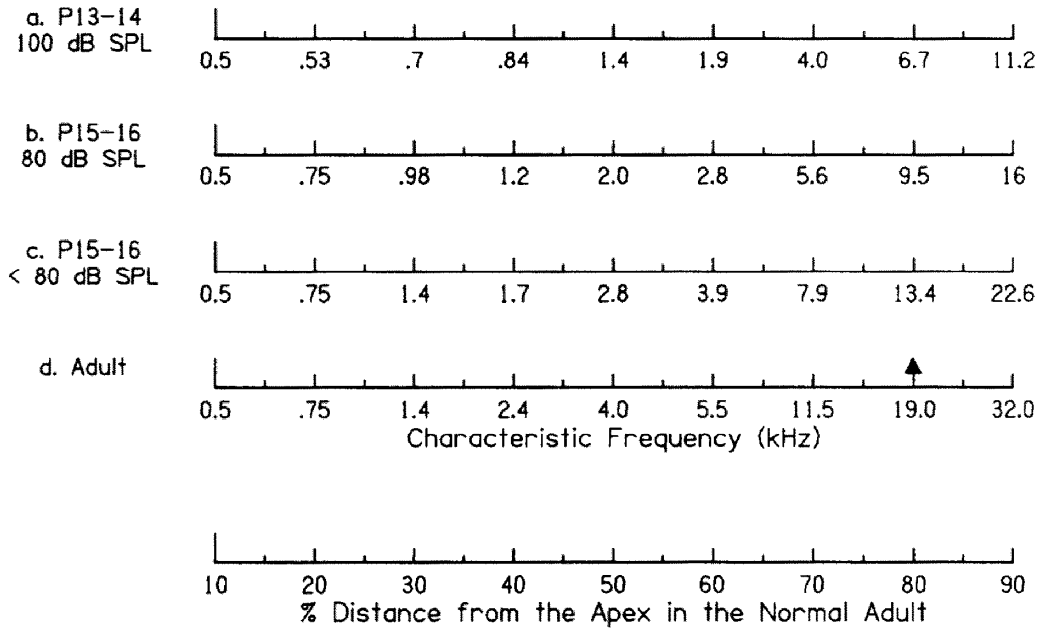

Fig. 12 Hypothetical place code maps for the developing gerbil. The characteristic frequency is given for a fixed place along the cochlear partition. (d) Shows the normal adult gerbil place code. In this case, both the active and passive systems are normal and functional. (c) Shows the place code map in the absence of functional active elements, i.e. nonfunctional outer hair cells. The result is that the CF of a given place is 1/2 octave lower than in the normal adult, except in the apical 30–35% of the cochlea. (b) Shows the case where the basilar membrane is less stiff and more dense than normal, in addition to outer hair cells being nonfunctional, and stimulus levels must be increased to 80 dB SPL to elicit a response. The CF of a given place is now one octave less than in the normal adult. (a) is the same as (c) except that the stimulus level is now 100 dB SPL and CF is shifted another 1/2 octave lower.

tunately they did not offer more detailed information concerning these changes.

Fig. 12b shows the situation when OHCs are non-functional and the stimulus level is raised to 80–85 dB SPL. The peak of basilar membrane displacement shifts another 1/2 octave lower. Another 20 dB increase in stimulus level could shift the apparent CF down yet another 1/2 octave, as illustrated in line a of Fig. 12. Hypothetically, if one point on the basilar membrane, such as that tuned to 19 kHz in the normal adult, is being monitored it could respond maximally to a 100 dB SPL signal 1.5 octaves lower (i.e. 6.7 kHz) than its normal CF in a P13–14 animal with an immature basilar membrane (slightly less stiff and more dense than in a normal adult) and with a non-functional basilar-membrane-outer-hair-cell system. As the basilar membrane matures, its CF shifts to a higher frequency due to both factors: 1) it takes less intensity to obtain the same hair cell response, thus reducing the 'basal shift'; and 2) the outer hair cell, active processes develop.

This scheme provides a good fit to the gerbil LSO tuning data of Sanes et al. (1989), the 8th nerve tuning curve data of Echteler et al. (1989), and the SP and AP data of Yancey and Dallos (1985). In their Fig. 8. (page 442), Sanes et al. reported that at the 80th percentile from the apical projection the predicted CF for adults is 19 kHz, whereas it is 7 and 12 kHz for the P13–14 and P15–16 groups, respectively. The former corresponds to a shift of −1.4 octaves, whereas the latter is −0.6 octaves. Thresholds for P13–14 animals are usually about 100 dB SPL or greater versus 80 to 60 dB for P15–16 and 5–20 dB SPL for adults. The filled triangle on line d in Fig. 12, is at the 19 kHz place for our hypothetical normal adult gerbil cochlea. The shifts in CF of that place seen in lines a,b and c correspond well to those reported by Sanes et al.

Development of responses to low vs high frequencies

In addition to accounting for ontogenetic place code shifts, the development of active elements

also may underlie what appears to be a greater sensitivity to low frequencies in developing animals. The absence of mature outer hair cell processes in young animals would result in broadened tuning. This results in a greater population response to low frequencies than normally found in adult animals because larger areas of the cochlea are contributing to the response. It is likely that initial behavioral and neural responses to low frequencies in developing animals are mediated by mid and basal areas of the cochlear partition; that is, by what will become the 'low frequency tails' for neural units innervating these regions. As active processes mature, the bandwidth of a particular place on the basilar membrane decreases, and there is a greater filtering of low frequencies. Finally, because of anatomical and functional differences in the apical and basal cochlea, place code shifts are not seen for low frequencies. Thus, the development of $2f_1-f_2$ ADPs should correlate primarily with development of high-frequency sensitivity and with the age at which an animal can hear up to a particular frequency with adult-like sensitivity, but not necessarily with low-frequency sensitivity. In fact, the time course of the maturation of the $2f_1-f_2$ ADP seen in this study and the maturation of CM or SP upper cut-off frequency observed by Harris and Dallos (1984) and Yancey and Dallos (1985) are quite consistent. That is, at P18–19 the CM cut-off frequency of the middle of the basal turn is approximately 13.0 kHz, the same value as they observed for adult animals. At this age, we found that $2f_1-f_2$ ADPs reached the adult range.

Finally, an attractive feature of the proposed model is that it makes specific predictions which are easily tested. First, as noted above, the model predicts that the maturation of sensitivity and frequency selectivity for high frequency sounds should coincide with the ontogeny of $2f_1-f_2$ ADPs evoked by a given f_2 frequency. Comparisons of our data with published reports on the ontogeny of physiological processing at the level of the cochlea and the brainstem auditory nuclei shows a rough temporal correspondence. However, a much better test of this prediction would be to examine multiple measures in the same animals at various maturational stages.

A second prediction of the proposed model is that manipulations that are known to effect active processes, such as hypoxia or cooling, will have relatively little effect on ADPs or cochlear output in young animals. In addition, their effectiveness on responses to specific frequencies will follow the maturational timetable of $2f_1-f_2$ ADPs. To our knowledge, experiments examining the vulnerability of otoacoustic emissions as a function of age and frequency have not been attempted.

The third and most robust prediction of our model is that the maturation of the active process and its contribution to cochlear processing can be directly examined at any age by comparing $2f_1-f_2$ ADPs in live animals with their characteristics immediately after death. The model predicts, in agreement with anatomical data, that the physiologically vulnerable active process matures along a generally basal to apical gradient. At any point in the maturational process, degradation of ADP input-output functions immediately after death represents the contribution of this active process. Preliminary data presented in Fig. 3 are consistent with our prediction: in young animals there is a relatively minor change (~ 5 dB) while in older animals the input-output functions are shifted 30–40 dB for stimuli below 65 dB SPL. However, a more systematic examination of this prediction is warranted and is currently being undertaken.

In summary, the development of $2f_1-f_2$ ADPs from the gerbil cochlea suggests that the development of active processes is a primary mechanism underlying the development of frequency selectivity and shifts in the place code map. This hypothesis makes several specific and testable predictions which are in need of direct evaluation in a variety of species. In addition, the model implies that otoacoustic emissions can be used as a non-invasive tool with which to examine the ontogeny of active elements contributing to auditory information processing.

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