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Ontogenetic Changes in Frequency Mapping of a Mammalian Ear

Abstract. Cochlear microphonic iso-response functions reported here suggest an explanation of frequency-dependent changes in hearing sensitivity during early development. The work is a direct demonstration of developmental changes in the spatial frequency map of the mammalian hearing organ. Intracochlear recordings from the midbasal turn in a series of age-graded gerbils reveal a progressive increase in best frequency, spanning approximately two octaves, from the time of onset of function until adultlike responses are seen. It is, therefore, suggested that ontogenetic changes in the cellular structure of the organ of Corti contribute to an age-dependent shift in micromechanical response.

Puzzling discrepancies are evident during development between sequential anatomical changes in the cochlea on the one hand and concomitant physiological measures on the other. The structural maturation of the cochlea progresses from the basal (high-frequency) end of the structure toward its apical (low-frequency) end. For example, differentiation and innervation (1-5) of the mammalian cochlear receptor cells first occur in the basal turn and progress apically. The same sequence has been reported for the morphological development of the supporting structure of the organ of Corti, including the opening of the tunnel of Corti, spaces of Nuel, and the inner spiral sulcus (1, 3, 6-9). In contrast, behavioral and physiological measures of auditory abilities at the time of the onset of function indicate that responses are first elicited by stimuli in the low- to middle-frequency range for any particular species studied (9-11). Since it has been well established that in the adult the basal segments of the cochlea preferentially respond to high-frequency stimuli while more apical portions are tuned to lower frequencies (12, 13), there seems to be a contradiction between the anatomical and the functional data. It has been suggested that selective attenuation of high frequencies by the immature middle ear may explain this conflict (14). Several investigators proposed that developmental changes in cochlear, in addition to middle-ear, mechanics underlie the discrepancy (3, 11, 15). Most nota-

bly, Rubel and his colleagues (9, 16) have suggested, on the basis of work with avian ears, that the immature basal portion of the cochlea is maximally responsive to low or low-middle frequencies. It is assumed that the place of maximum

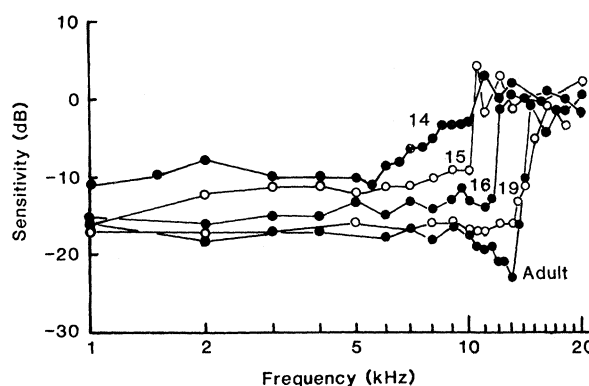


Fig. 1. Examples of sensitivity patterns obtained in individual gerbils as a function of stimulus frequency. The age of the animal in days is indicated on each curve. These functions are obtained as follows. An intracochlear electrode (24 μ m diameter tungsten wire insulated with glass) was placed in the scala tympani of the first (basal) cochlear turn. Another electrode, silver wire, was placed on the round window membrane. The indifferent electrode was a common location on the metal head-holding

device. Electrical signals were amplified in separate wide-band amplifiers, displayed on an oscilloscope, and measured with a 3-Hz bandwidth wave analyzer. Sound was delivered to the animal's ear by a headphone assembly (Beyer), an integral part of which was a probe-tube microphone for measuring sound pressures in the immediate vicinity of the eardrum. The data were obtained in terms of the sound pressure level necessary to elicit a criterion (1 μ V) CM response at any given frequency. Sensitivity was computed as the decibel difference between the first turn and the round window readings. Thus a negative number indicates greater sensitivity (lower sound pressure level) at the intracochlear recording site. Note that all functions, including that depicting adult behavior, show a high-frequency plateau. The level of this flat portion is largely determined by the noise level of the recording apparatus. Data points within this plateau for any animal were averaged, and the resulting value was arbitrarily designated as 0 dB. Thus the plots depict data normalized to the level of this high-frequency plateau. Before data collection, the animals were anesthetized (5 to 20 mg of ketamine per kilogram of body weight, dose increasing with age) and the auditory bulla was opened from a basolateral approach (19). In adults a tracheal cannula was placed, but in pups the natural airway was maintained. During surgery and experiments the animals were kept warm with the aid of a water blanket. In adults the hole admitting the intracochlear electrode was drilled by hand through the bony capsule (13). In young animals (up to 14 days) the capsule is cartilaginous and drilling is not possible. In these cases the hole was made by gently piercing the capsule with a fine needle.

sponse characteristics of the active recording location. The frequency dependence of the middle ear and the response properties of the sound system should influence the intracochlear and round-window recordings approximately equally. Moreover, the round-window recordings obtained from the extreme basal end of the cochlea have the widest bandwidth of any recording site (18). Thus the difference between the two iso-response curves represents the frequency mapping properties of the midbasal turn. These differential CM iso-response functions typically show a relatively flat or increasing sensitivity from low to high frequencies. Then, depending on the electrode location (in the adult), a particular cut-off frequency is passed, whereupon for higher stimulus frequencies sensitivity drops precipitously. We used this cut-off frequency as an estimate of the best frequency of the recording location.

The data from the first cochlear turn in the developing gerbil are straightforward and consistent. The curves shown in Fig. 1 are representative of the trend we have observed in the first 2 weeks after the onset of measurable responses. By our methods CM responses can be first seen in the gerbil at 12 to 13 days after birth (hereafter referred to as days). This is in agreement with the observations of Finck *et al.* (20) and Woolf and Ryan (21). A first-turn function with a cut-off frequency of 13.0 kHz for a young adult is shown for comparison. The 14-day curve illustrates the first pattern to emerge. This function has a shallow high-frequency slope with a cut-off frequency well below that seen in the adult. By 19 to 20 days the cut-off frequency progressed into the adult range. The relation between cut-off frequency and age for a sample of 41 gerbils is summarized in Fig. 2. These data illustrate a trend for best frequency to increase with age at the first-turn recording site. Although the first responses could be measured at 12 days, in recordings from two 12-day and one 13-day animals, an accurate cut-off frequency was impossible to determine. Nevertheless, in these animals evoked activity was restricted to below 5 kHz. Once a cut-off frequency could be clearly discerned (14-day curve in Fig. 1) it gradually progressed to higher values over the next 7 to 10 days. Concurrently the high-frequency slope steepened and sensitivity increased. Fully adultlike responses were seen for some animals as young as 19 days, although these trends seem to continue, albeit at a decreasing rate, beyond day 19. Best frequencies for adults were measured in the approxi-

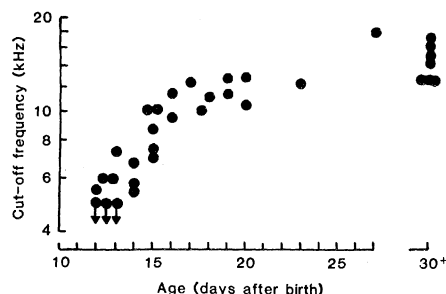


Fig. 2. Cut-off frequency as a function of age. Cut-off frequency was determined from plots of the type shown in Fig. 1. For example, the cut-off for the illustrated 19 day animal is 13.0 kHz. In three very young animals a clearcut cut-off frequency could not be established, but electrical activity was confined to below 5 kHz. These are indicated by data points with arrows. The adult population consists of animals 30 days or older; they are all plotted at the 30+ point.

mately half-octave range from 12.5 to 18.5 kHz (22). Thus an overall shift in best frequency of the recording location in the first turn between 12 days and adulthood exceeds two octaves.

These data lend validity to the hypothesis of a tonotopic shift during development. It seems accurate to characterize the midbasal region of the cochlea as developing sensitivity to stimuli of progressively higher frequency after the onset of cochlear function. In other words, the cochlear frequency map changes during development. It is conceivable that the observed changes result from an age-related change in the relative recording location due to continued growth of the cochlea during this period. At birth in the cat (3) and rabbit (23), the bony labyrinth has attained its final dimensions, although the ear is still growing in the mouse (5). One may estimate for the adult the cochlear distance corresponding to our two-octave frequency shift. This is computed as 3 mm on the basis of available mapping data (19). It seems unlikely that gross dimensional changes of this magnitude (25 percent of the total adult length) would occur in the basal turn during our time window.

It is more likely that ontogenetic changes in the microscopic structure of the organ of Corti occurring during this time (1, 2, 7, 8) influence the local mechanical response to high-frequency stimuli. Many synchronized and partially overlapping morphological, biochemical, and physiological changes in receptor, supporting, and neural elements occur during these early stages of development. As an example, several investigators (1, 5, 7) have described changes in the cellular structure of the cochlear partition of the mouse that occur from 5

to 15 days. Since the timetable of development of cochlear function and behavioral responses in the mouse (9, 24) resembles that of the gerbil, their observations may be relevant to the interpretation of our results. The adult width of the basilar membrane is established by 5 days. At this time, however, an epithelial cell layer exists on the scala tympani side. This tympanic layer is reduced in thickness and disappears shortly after 15 days. The basilar membrane concurrently gains bundles of radially oriented filaments, and at the same time the entire partition loses some of its cellular structure, leading to the opening of the inner spiral sulcus and spaces of Nuel. The pillar cells are also undergoing alterations as their filaments increase in number and organize into bundles; the bases of the pillar cells separate, forming the tunnel of Corti. These morphological changes indicate a progressive decrease in mass and an increase in stiffness. Both of these factors can be assumed to shift local mechanical resonance from lower to higher frequencies.

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Leukotriene B₄ Produces Hyperalgesia That Is Dependent on Polymorphonuclear Leukocytes

Abstract. *Leukotriene B₄, at the same intracutaneous doses as bradykinin, reduced the nociceptive threshold in the rat paw. The mechanism of leukotriene B₄-induced hyperalgesia was distinguished from that of the hyperalgesia elicited by prostaglandin E₂ and bradykinin by its dependence on polymorphonuclear leukocytes and independence of the cyclooxygenation of arachidonic acid.*

The most characteristic sensory abnormality associated with inflammation is tenderness or hyperalgesia. Hyperalgesia develops when nerve fiber terminals of polymodal nociceptors are sensitized by mediators of inflammation (1), such as prostaglandin products of the cyclooxygenation of arachidonic acid. Prostaglandins produce hyperalgesia at low concentrations (2) without evoking pain (3). Nonsteroidal anti-inflammatory drugs (NSAID's) reverse hyperalgesia, in part by inhibiting the biosynthesis of prostaglandins (4). However, the failure of NSAID's to reverse consistently the hyperalgesia of inflammation at concentrations that suppress prostaglandin generation (5) and the finding that many noninjurious stimuli can elicit the generation of prostaglandins without producing hyperalgesia (6) both suggest that inflammatory mediators other than prostaglandins contribute to the sensitization of nociceptors.

Leukotriene products of the NSAID-resistant 5-lipoxygenation of arachidonic acid are potent stimuli of many components of inflammation. Such lipoxygenase products mediate hyperalgesia in animals (7) and humans (8). We now report that leukotriene B₄ (LTB₄) produces hyperalgesia, in rat paws, that is not dependent on the integrity of the cyclooxygenase pathway but does require polymorphonuclear leukocytes that recognize specifically and respond functionally to LTB₄.

We defined hyperalgesia as a decrease in nociceptive threshold in male Sprague-Dawley rats (200 to 250 g) and quantified it by determining the decrease in local pressure (measured in grams per square centimeter) required to evoke

withdrawal of the paw (9). We increased the pressure applied to the dorsum of the paw linearly with time and recorded the pressure when the rat withdrew its paw. This reflex correlates well with hyperalgesia evoked in humans in response to intradermal injections of noxious agents and with responses to analgesic compounds (10). After measurement of the baseline nociceptive threshold, a test or control substance (10 μ l) was injected intradermally in the dorsum of one hind paw. The intensity of the hyperalgesia, at the site of the injection, produced by the substance at each time was expressed as the percentage decrease in the nociceptive threshold from the baseline value.

The substances used were synthetic bradykinin (Sigma Chemical Company, St. Louis, Missouri), prostaglandin E₂ (PGE₂) (Upjohn Company, Kalamazoo, Michigan), and LTB₄ and LTD₄ (Dr. J. Rokach, Merck-Frosst Co., Dorval, Canada). Native LTB₄, 5(S),12(S)-dihydroxy-

droxyeicosa-6,8,10-*trans*-14-*cis*-tetraenoic acid [12(S)-6-*trans*-LTB₄], and 12(R)-6-*trans*-LTB₄ were extracted from supernatants of suspensions of human neutrophils, incubated with arachidonic acid (50 μ g/ml) and 2 μ M ionophore A23187, and purified by high-performance liquid chromatography (HPLC). The incubation of 1 mg of arachidonic acid with 10,000 units of purified soybean 15-lipoxygenase (Sigma) in 2 ml of 0.2M sodium borate (pH 8.8) for 30 minutes at 37°C generated a mixture of 15-HETE and diHETE's, from which 8(S), 15(S)-dihydroxy-5, 11-*cis*-9, 13-*trans*-eicosatetraenoic acid (8,15-diHETE) was extracted and isolated by HPLC (11). All compounds tested for their ability to induce hyperalgesia were dissolved in phosphate-buffered saline. Indomethacin (Sigma) was dissolved in sodium bicarbonate (2 g/100 ml) titrated to pH 7.2 with sodium phosphate and was administered intravenously (2 mg/kg) 20 to 30 minutes before intradermal injections.

Polymorphonuclear leukocytes were depleted by giving hydroxyurea (Squibb Company, Princeton, New Jersey) or methotrexate (Lederle Company, Puerto Rico) to rats intravenously for 3 days (12). The administration of hydroxyurea, 200 mg/kg on day 1 and 100 mg/kg on days 2 and 3, or methotrexate, 2 mg/kg on day 1 and 1½ mg/kg on days 2 and 3, eliminated polymorphonuclear leukocytes, other granulocytes, and mononuclear leukocytes from the circulation on days 4, 5, and 6 as assessed with Wright-stained slides of peripheral blood. Only rare polymorphonuclear leukocytes were observed on each of two slides of peripheral blood prepared from each rat treated with hydroxyurea or methotrexate. No mortality or morbidity was observed in rats receiving either drug. Since similar results were obtained with both agents, and all groups contained equal numbers of rats treated with either

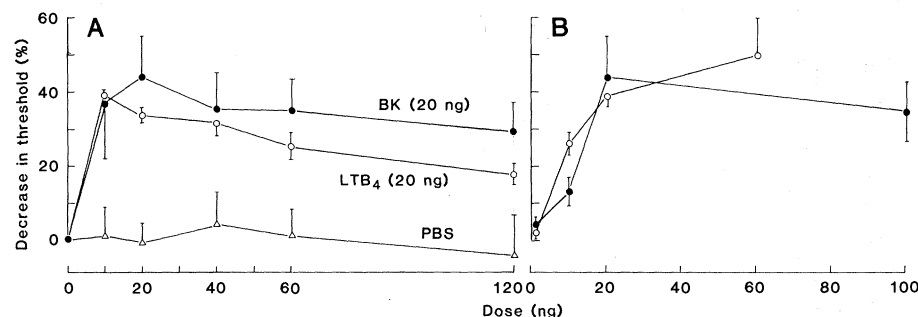


Fig. 1. (A) Time course of the effects of LTB₄ on nociceptive pressure thresholds in the rat paw. Leukotriene B₄, BK, and the phosphate-buffered saline (PBS) vehicle were injected intradermally in the dorsum of the paw. Each point and bracket represents the mean $\pm$ standard error of the results of studies of six rats. (B) Concentration dependence of the effect of BK and LTB₄ on nociceptive pressure thresholds. Measurements were made 20 minutes after the intradermal injection of BK (●) and LTB₄ (○). Each point represents an average of six experiments.