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Frequency tuning of mechanical responses in the mammalian cochlea

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The search for mechanisms responsible for the high sensitivity and sharp frequency tuning of first-order auditory neurons has produced surprising results. The cochlea, the mammalian auditory receptor, responds to acoustic stimuli with a sharply frequency tuned, nonlinear vibration that enhances low level stimuli, but generates appreciable distortion. This highly sensitive mechanical response is achieved by an electro-mechanical feedback process in which outer hair cells reinforce cochlear motion at low stimulus intensities.

Key words: *auditory, basilar membrane, cochlea, frequency tuning.*

INTRODUCTION

Auditory stimuli usually are complex mechanical vibrations containing many frequency components that may range, for humans, from about 20 Hz to 20 kHz. The auditory system is able to discriminate among these components and to perceive them even though those with the highest frequencies exceed the time resolving capabilities of the nervous system. The responses to sound of auditory nerve fibers are highly tuned in frequency: each nerve fiber is most sensitive to stimuli at a certain frequency, its characteristic frequency (CF), and requires increasingly higher intensities to produce the same response as the stimulus frequency departs from its CF. Thus, at the very first step of auditory processing, in the cochlea, mechano-electrical transduction is accompanied by a process of spectral analysis separating auditory stimuli into their frequency components in a way similar to that done mathematically by Fourier analysis. This peripheral frequency analysis provides a mechanism by which the auditory system is able to discriminate the high-frequency components of sound.

The cochlea is an inner ear organ exquisitely adapted to detect mechanical vibrations. In man, as in all mammals, the cochlea consists of a long membranous double tube coiled in the shape of a snail and completely surrounded by bone. Its two main cavities, the scalae vestibuli and tympani, are filled with perilymph, a liquid similar to cerebrospinal fluid. In between these two compartments there is a third cavity, the scala media, filled with endolymph (see Fig 1). Endolymph, a high K^+-concentration fluid, has a positive electrical potential with respect to perilymph (endocochlear potential). Within the scala media, on the basilar membrane, lies the organ of Corti, a structure that holds the cells responsible for mechano-electrical transduction, the hair cells. This arrangement leaves the organ of Corti in an ideal position to vibrate in response to mechanical stimuli that produce pressure differences between scalae vestibuli and tympani. The transverse motion of the organ of Corti produces a radial movement of the stereocilia at the top of

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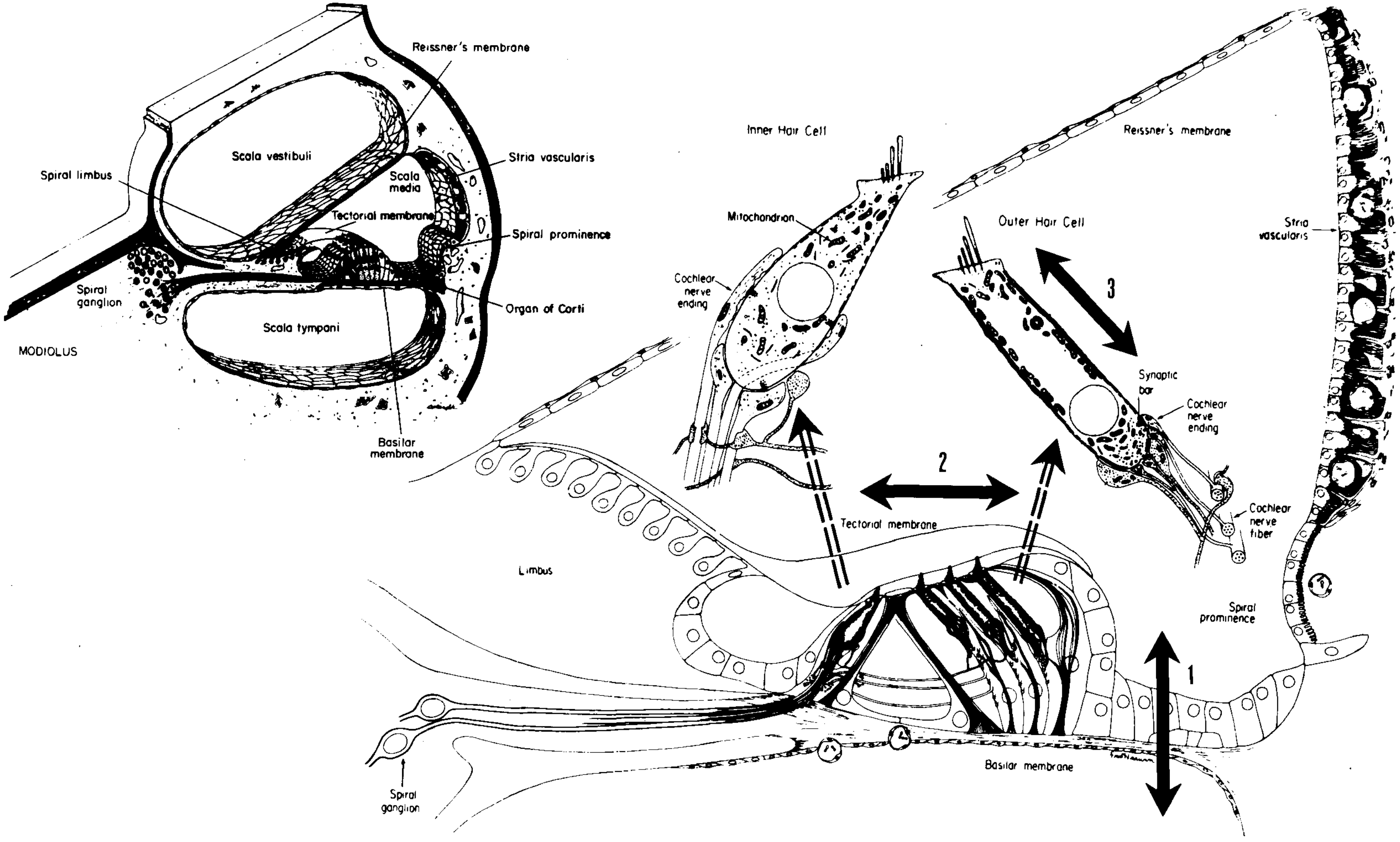

Fig 1. Inset. Illustration of a cross-section of the cochlea depicting the three scalae, the basilar membrane and the organ of Corti. (Reproduced from Fawcett, 1986). **Main figure.** Drawing of a cross-section of scala media in the second turn of the Guinea pig cochlea including enlarged views of an inner and an outer hair cell. The solid arrows indicate the direction of: 1) vibration of the basilar membrane and organ of Corti, 2) radial movement of the stereocilia of hair cells producing forward transduction and 3) active longitudinal movement of outer hair cells produced by reverse transduction. (Modified from Smith, 1975).

the hair cells which, in turn, gates cationic channels, thus generating transduction currents and receptor potentials in the hair cells (for a review on hair cell transduction see Hudspeth, 1989).

Upon reaching the eardrum or tympanic membrane sounds are transmitted to the cochlea by the middle ear ossicles. The last one of the ossicles, the stapes, vibrates as a piston and impresses on the perilymph of scala vestibuli pressure changes that displace the basilar membrane with the organ of Corti. Basilar membrane motion propagates as a wave, that travels from the base of the cochlea toward its apex, reaching its maximum amplitude at different sites depending on the stimulus frequency (see Fig 2).

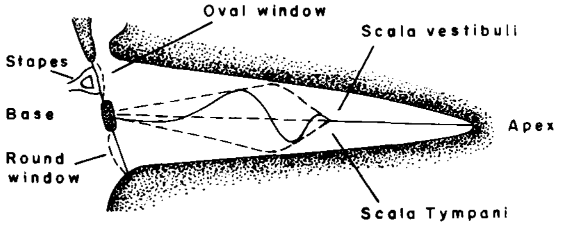

Fig 2. Simplified diagram of the uncoiled cochlea showing scalae vestibuli and tympani (scala media has been omitted). The solid line represents the instantaneous pattern of displacement of the basilar membrane (and organ of Corti) in response to a middle frequency tone. The dashed lines illustrate the envelope of the travelling waves at this particular frequency. Vertical displacements of vibration are highly exaggerated. Higher frequency stimuli reach the maximum of their envelopes at more basal locations and lower frequency stimuli at more apical ones.

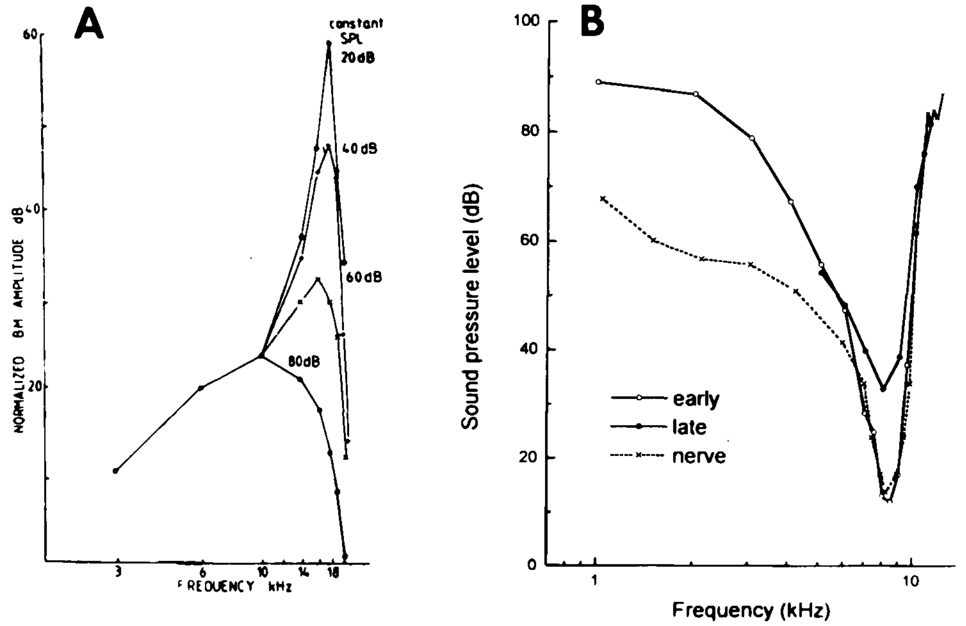

Fig 3. A. Frequency responses of a basal point in the Guinea pig cochlea. Each curve shows basilar membrane amplitude of vibration in response to stimuli at various frequencies and at four constant sound pressure levels. For comparison the curves have been normalized to a stimulus intensity of 20 dB sound pressure level, by reducing the responses at each intensity level by the dB value exceeding 20 dB SPL (e.g., the response to 80 dB SPL has already been reduced by 60 dB to make it comparable to that measured at 20 dB SPL). The striking loss in sharpness of tuning and sensitivity of the curves as stimulus intensity increases shows the strong nonlinearity of the cochlear mechanical response. At frequencies lower than 10 kHz, the curves superimpose displaying a linear response. (Reproduced from Johnstone *et al*, 1986). **B.** Basilar membrane and auditory nerve tuning curves of a point at the basal turn of the chinchilla cochlea. Solid lines represent basilar membrane isovelocity tuning curves obtained in one chinchilla, early (filled circles) and late (open circles) in the experiment. These curves display the stimulus sound pressure required at each frequency to obtain a constant basilar membrane velocity response (0.1 mm/s). The dashed line indicates an auditory nerve tuning curve, obtained as the average of single-unit rate-threshold curves from several fibers with CF around 8.5 kHz. The curves demonstrate a drastic loss of sharpness and sensitivity around CF due to deterioration in the physiological state of the cochlea. They also show the remarkable similarity in tuning between the mechanical and auditory nerve responses. (Data reploted from Robles *et al*, 1986).

Mainly owing to the increase of compliance of the basilar membrane from base to apex, high-frequency tones attain maximum amplitude at the base and low-frequency tones at the apex of the cochlea. Early measurements found that cochlear mechanical responses were frequency tuned, but the tuning was much too broad to account for the frequency selectivity of auditory nerve fibers and of psychophysical measurements (von Békésy, 1960). A turning point in cochlear physiology was the discovery that basilar membrane responses display nonlinear properties that are vulnerable to cochlear injury and disappear after animal death (Rhode, 1971).

FREQUENCY TUNING OF MECHANICAL RESPONSES

Experiments performed in anesthetized animals with minimal cochlear damage have corroborated the existence of the nonlinearity in basilar membrane motion and have shown that cochlear mechanical responses are as sharply frequency tuned as those obtained in cochlear nerve fibers (Sellick *et al*, 1982; Robles *et al*, 1986; Nuttall *et al*, 1991; Cooper and Rhode, 1992; Murugasu and Russell, 1995). Figure 3A displays normalized basilar membrane vibration amplitude measured at the basal turn of the Guinea pig cochlea in response to stimuli at four intensity levels and at several frequencies (Johnstone *et al*, 1986). All of the frequency response curves exhibit a band-pass characteristic, which becomes narrower as stimulus intensity is decreased. The response at the lowest stimulus intensity (20 dB of sound pressure level) has a sharply tuned sensitivity maximum at the CF for the cochlear location under study. The curves for higher intensities have broader maxima and much lower sensitivity (Fig 3A shows a sensitivity decrease of about 40 dB for a 60 dB increase in stimulus level). This frequency-dependent loss of sensitivity as a function of stimulus intensity is a fundamental nonlinear feature of the cochlear mechanical response. By contrast, at frequencies well below and above CF the response is linear, as shown by the superposition of the sensitivity curves measured at different stimulus intensities. Figure 3B shows the dependency of cochlear tuning and sensitivity on the physiological condition of the cochlea. The figure depicts basilar membrane tuning curves (solid lines) measured at a basal point in the chinchilla cochlea, early and late in the experiment (Robles *et al*, 1986). The curves show a drastic loss in frequency tuning and sensitivity restricted to the frequencies around CF (8.5 kHz), produced by deterioration of the cochlea. Figure 3B also includes a frequency-threshold tuning curve (dashed line) recorded from auditory nerve fibers (with appropriate CFs) at the base of the chinchilla cochlea. The comparison of the early mechanical curve with the neural tuning curve shows that, at the frequencies around CF, both tuning curves are remarkably similar. These *in vivo* measurements have shown that, in mammals, the sharp frequency tuning and high sensitivity of the auditory nerve at low stimulus intensities is determined by the cochlear mechanical response. They have also demonstrated that this mechanical response includes a physiologically vulnerable component that enhances basilar membrane motion at frequencies around CF.

ACTIVE RESPONSE OF THE COCHLEA

Even before the sharply tuned mechanical response of the cochlea was well established, there was an unexpected experimental finding that shook the foundations of cochlear physiology. This finding was that the cochlea in humans and animals can produce evoked or even spontaneous acoustic emissions, recordable -as pressure changes- at the external ear canal (Kemp, 1978). The existence of these otoacoustic emissions, particularly the spontaneous variety, implied the existence in the cochlea of oscillating elements that deliver energy to the cochlear structures. Further, some theoreticians working with mathematical models of the cochlea found it impossible, using only passive elements, to match the sharp tuning and high sensitivity of basilar membrane responses measured in *in vivo* experiments (Davis, 1983; Neely and Kim, 1983;

Diependaal *et al*, 1987). These experimental and theoretical findings suggested that the cochlea achieves its extraordinary performance by means of active elements that, at low stimulus levels, amplify cochlear motion. This revolutionary idea actually was not new, having been first suggested by Gold (1948) almost fifty years ago. Gold proposed that, in order to accomplish the high frequency resolution observed in auditory psychophysical tests, there should be a feedback process by which energy is supplied to the cochlea to counteract the losses produced by the viscosity of the fluids in which the organ of Corti vibrates.

There are several reasons that make outer hair cells, among the components of the organ of Corti, the natural candidates to perform this active mechanical function. 1) About 95 % of the afferent cochlear innervation originates at inner hair cells, while outer hair cells receive mostly efferent innervation. 2) Outer hair cells loss or their alteration, by means of drugs or efferent stimulation, can modify the cochlear response (transduced mainly by the inner hair cells). 3) Distortion-product otoacoustic emissions, generated within the cochlea (under two-tone stimulation, see following section) and recorded at the ear canal, can be altered by changes in endocochlear potential and by stimulation of the efferent system (Mountain, 1980), thus showing that outer hair cells can modify the mechanical response of the cochlea. A more direct proof of this motor capacity of outer hair cells in the *in vivo* cochlea was provided by the demonstration that systemically injected furosemide, a diuretic that produces a rapid and reversible decrease of endocochlear potential, reduces the tuning and sensitivity of the cochlear mechanical response to sound (Ruggero and Rich, 1991).

Two possible mechanisms have been proposed by which outer hair cells could exert forces on the organ of Corti, modifying its mechanical response: the active movement of their stereocilia and changes in their somatic length. Oscillatory movements of stereocilia have been observed in cochlear hair cells of reptiles (Crawford and Fettiplace, 1985) and amphibia (Howard and Hudspeth, 1987). Even though these movements have not been reported in mammals, this is an attractive hypothesis since contractile proteins have been identified in stereocilia and elsewhere in the apical region of hair cells. The second mechanism is based on the discovery that isolated outer hair cells *in vitro* elongate and contract under electrical or chemical stimulation (Brownell *et al*, 1985). Isolated outer hair cells show longitudinal electromotility with a tonic (slow) component and a fast component that has been observed up to frequencies in the range of kiloHertz (Ashmore, 1987; Santos-Sacchi, 1992; Dallos and Evans, 1995). Consequently, it has been suggested that the outer hair cell mechanical feedback could work in the cochlea in a cycle by cycle basis (as an AC motor) or, for high stimulus frequencies by a tonic displacement (as a DC motor). Indeed, changes of receptor potential induced by hair bundle deflection (forward mechano-electrical transduction) can produce somatic length changes in isolated outer hair cells (backward electro-mechanical transduction, Evans and Dallos, 1993). The direct stimulus to the motile response in outer hair cells seems to be the local transmembrane potential (Santos-Sacchi and Dilger, 1988), which could be detected by charged voltage-sensing particles; the movement of these hypothetical particles is consistent with the existence in outer hair cells of nonlinear capacitive currents, similar to the gating currents measured in ionic channels (Ashmore, 1989; Santos-Sacchi, 1991). Recent experiments using suction and patch-clamp pipettes in isolated outer hair cells indicate that the cellular motor mechanism should be one based on the additive effects of many independent motor units (Dallos *et al*, 1991). These motor units could be molecules associated with the plasma membrane (Kalinec *et al*, 1992) or submembrane cortical structures.

There are still questions as to whether the electromotile response observed in isolated outer hair cells can account for the active component of cochlear mechanical responses measured *in vivo*. In the latter condition the outer hair cells are anchored within the organ of Corti and it is not yet known how efficiently their motion can be transferred to other cochlear structures. However, new experiments have brought the results obtained in isolated hair cells closer to the *in vivo* measurements: electrical currents applied across the organ of Corti can produce place-specific basilar membrane displacements in isolated cochleas (Mammano and Ashmore, 1993), as well as frequency tuned basilar membrane responses in experiments *in vivo* (Xue *et al,* 1995).

COCHLEAR DISTORTIONS

One of the consequences of the nonlinearity of basilar membrane motion is the generation of distortion in the cochlear response to auditory stimuli. Auditory distortion was already known to eighteenth century musicians, who reported that subjects listening to pairs of tones could hear additional tones not present in the stimulus (Jones, 1935). Among these two-tone distortion products, those with frequencies $2f_1 - f_2$ and $f_2 - f_1$ (for primaries f_1 and f_2, $f_2 > f_1$) have been extensively studied in psychophysical experiments and found in electrophysiological recordings from auditory nerve fibers and hair cells. Two-tone distortion products have also been observed as otoacoustic emissions at the ear canal. Recently distortion products have been shown to be intrinsic components of the normal basilar membrane response and to propagate along the cochlea as stimulus tones (Robles *et al,* 1990, 1991; Nuttall *et al,* 1990; Rhode and Cooper, 1993). Figure 4 shows a frequency spectrum of basilar membrane motion, obtained in chinchilla, in response to two moderate intensity, simultaneous tones at frequencies f_1 and f_2. In addition to the peaks at the primary frequencies, the spectrum includes prominent peaks at various frequencies corresponding to combinations of the primary frequencies (Robles *et al,* 1991). The components at frequencies $2f_1 - f_2$ and $2f_2 - f_1$ in the spectrum reach amplitudes equivalent to distortions of about 7 % and 4.3 % respectively, of the primary responses. Although such high level of distortion in the mechanical response of the cochlea may be surprising, it may be the necessary result of the nonlinear process that nature has selected to increase cochlear sensitivity and frequency selectivity at low stimulus intensities.

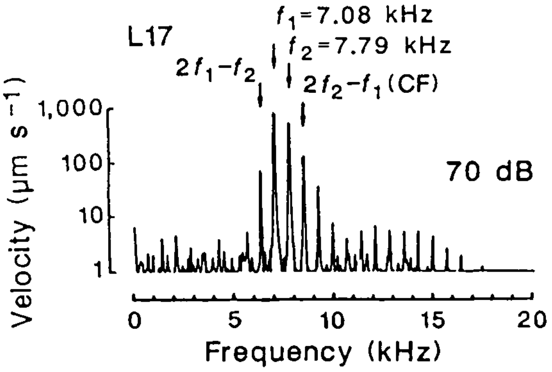

Fig 4. Frequency spectrum of basilar membrane responses to two-tone stimuli measured at the basal turn of the chinchilla cochlea. The spectrum displays peaks at the primary frequencies (7.08 and 7.79 kHz), as well as at several frequencies corresponding to two-tone distortions. The spectral peaks at two of these distortions, extensively studied in psychophysical experiments ($2f_1 - f_2$ and $2f_2 - f_1$), have been indicated by arrows. (Modified from Robles *et al,* 1991).

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