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RECENT FINDINGS ON OUR AUDITORY SYSTEM: IT IS VERY SENSITIVE OWING TO THE MOTILITY OF SENSORY CELLS

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INTRODUCTION

The ears are paired sense organs, which collect, transmit, and detect acoustic impulses. Each of them is comprised of three main parts: the outer ear, middle ear, and inner ear. Traveling sound is focused into the external auditory canal by the pinna, causing vibration of the tympanic membrane and motion of the three ossicles in the middle ear. Their motion is transmitted to the cochlea of the inner ear. The mechanical motion of the basilar membrane in the organ of Corti of the cochlea is then transduced into encoded nerve signals in the cochlea, which are transmitted to the brain.

Even though the amplitude of the displacement of tympanic membrane vibrations is only a few nanometers when we speak in a low voice, we can clearly understand what is being said. This is due to cochlear amplification caused by the motility of outer hair cells (OHCs), which are located in the organ of Corti. The origin of this motility is believed to be associated with a membrane protein in the lateral wall of OHCs. The gene that codes for this protein has been identified and termed 'prestin.' Prestin has been found to be a direct voltage-to-force converter, which can operate at microsecond rates.

In my talk, firstly, actual measurement results of the tympanic membrane vibrations will be shown by video. Secondly, a dynamic animation of how the middle ear and cochlea function will be presented. Thirdly, the motility of the isolated OHC will be demonstrated, and the function of the OHCs, which behave like actuators of mechanical structures, will be discussed. Finally, images of prestin obtained by an atomic force microscope will be displayed.

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REMARKABLE SENSITIVITY OF THE EAR

Sound is energy that is transmitted by pressure waves in air and is the objective cause of the sensation of hearing. Figure 1 shows the auditory response area for humans [1, 2]. Sound within the dotted area is bounded on one side by the threshold of pain and on the other by the threshold of audibility as a function of frequency. The difference between these thresholds, i.e., the dynamic range, is quite wide, nearly 130 dB SPL at a frequency of 4.0 kHz. High-end recording equipment has a dynamic range of 90 dB. This means that our ears are much more sensitive than such equipment.

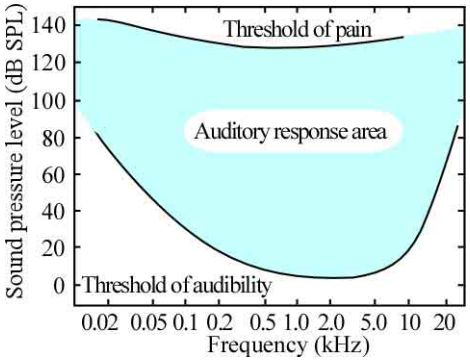

Figure 1. Auditory response area for humans. Sound within the dotted area is audible. This area is bounded on one side by the limits of tolerability of sound and on the other side by the limits of detectability. The difference between the two thresholds is quite wide.

OVERVIEW OF PERIPHERAL ANATOMY: OUTER, MIDDLE, AND INNER EARS

Figure 2 displays a computer-aided reconstruction of the human middle and inner ears, which was obtained from a fixed temporal bone extracted from a fresh cadaver. The relationship of size and location among the various components of the peripheral auditory system can be clearly understood. In humans, as shown in Fig. 3, the external auditory canal with a diameter of 7 mm and a length of 30 mm, which is slightly bent and elliptical, is terminated by a conical-shaped tympanic membrane with a diameter of 10 mm and a thickness of 0.1 mm. Three ossicles, namely, the malleus, incus, and stapes, are

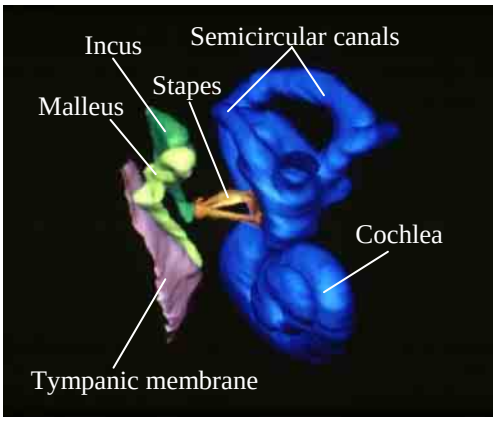

Figure 2. Computer-aided reconstruction of the human middle and inner ear, which was obtained from the temporal bone extracted from a fresh cadaver. The relationship of size and location among the various components can be clearly understood.

located in the tympanic cavity. The malleus is attached to the tympanic membrane, the incus lies between the malleus and the stapes, and the stapes is connected to the cochlea.

Figure 4 depicts the human cochlea with a length of 35 mm, which is spiral-shaped and has three fluid-filled compartments, i.e., the scala vestibuli, the scale media, and the scala tympani. They are separated by Reissner's membrane and the basilar membrane. The scala vestibuli and scala tympani contain perilymph, and the scala media contains endolymph. At the basal end, the scala vestibuli has an oval window, and the scala tympani has a round window. The base of the stapes, called the footplate, is sealed by a flexible ligament, and the footplate transmits the vibration of the middle ear to the fluid in the scala vestibuli.

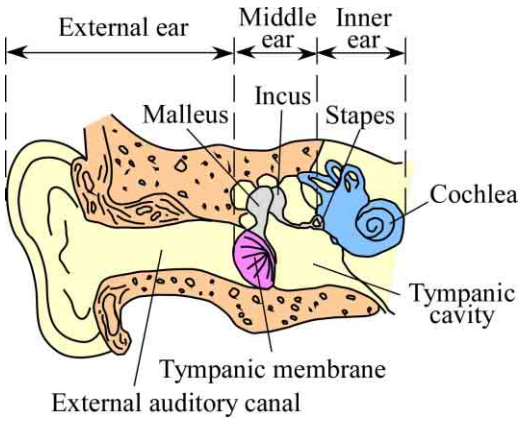

Figure 3. Human peripheral auditory system. Mammals always have three ossicles, namely, the malleus, the incus, and the stapes.

As shown in Fig. 5, the organ of Corti sits on the basilar membrane and contains two types of hair cells, i.e., the inner hair cells (IHCs) and the OHCs. There are approximately 3,500 IHCs and 12,000 OHCs in humans [3]. Hairlike structures, i.e., stereocilia, extend from the top of these cells. The organ of Corti is covered by the tectorial membrane and given rigidity by the pillar cells. There are three types of supporting cells, namely, Deiters', Hensen's, and Claudius' cells.

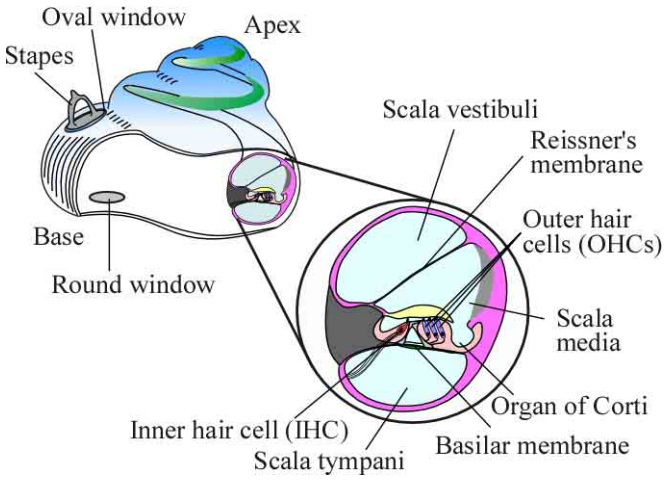

Figure 4. Human cochlea and its cross section. The cochlea has three fluid-filled compartments, which are divided by Reissner's membrane and the basilar membrane.

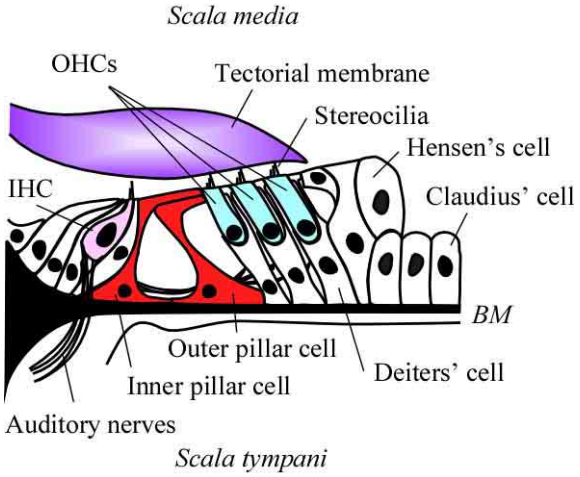

Figure 5. Structure of the organ of Corti. This organ sits on the basilar membrane. Two types of sensory cells, i.e., the inner hair cells (IHCs) and the outer hair cells (OHCs) are located in this organ.

ACOUSTICAL PROPERTIES OF THE MIDDLE EAR

An attempt was made to measure the vibratory responses of guinea pig tympanic membranes using time-averaged electric speckle pattern interferometry [4]. Figure 6 shows perspective plots of the displacement distribution of the left tympanic membrane vibrations when the displacement at each point reaches its maximum value. The amplitude of tympanic membrane vibrations is of nanometer order of two digits, i.e., 10-99 nm, when the sound pressure level of the stimulation is between 70 dB SPL and 85 dB SPL.

The three ossicles transmit sound vibrations from the tympanic membrane to the oval window of the cochlea. The main role of the middle ear is to match the low impedance of the air in the external auditory canal to the high impedance of the cochlear fluids. In other words, the middle ear is an impedance transformer. Without this function, much of the sound energy would be reflected. According to our numerical analysis [5], as shown in Fig. 7, the vibration mode of the ossicular chain varies with frequencies. However, as shown in Fig. 8, when the tympanic membrane vibrates, the ossicles basically rotate around the axis between the anterior malleal ligament and the posterior incudal ligament, and the umbo and stapes have a piston-like movement [6, 7]. The area of the tympanic membrane is much larger than that of the stapes footplate. The forces collected by the tympanic membrane, therefore, increase the pressure at the oval window. The arm of the malleus is larger than that of the incus, and this produces leverage, which increases the pressure and decreases the velocity at the stapes. By this mechanism, more than 30% of the sound energy reaches the cochlea.

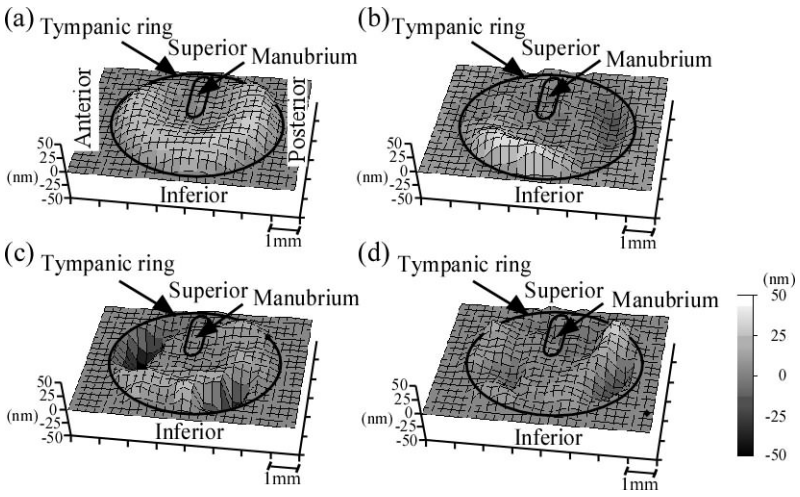

Figure 6. Perspective plots of the displacement distribution of the left tympanic membrane vibrations when the displacement at each point reaches its maximum value. (a) Frequency f = 1.0 kHz and sound pressure level P = 85 dB SPL. (b) f = 2.5 kHz and P = 70 dB SPL. (c) f = 3.0 kHz and P = 75 dB SPL. (d) f = 4.0 kHz and P = 75 dB SPL. At the frequency of 1.0 kHz, the whole tympanic membrane vibrates in phase. The maximum displacement amplitude is about 30 nanometers. At the frequency of 2.5 kHz, the tympanic membrane has two local maxima, one in the posterior portion and the other in the inferior portion. The number of the peaks increases and the vibration mode becomes complicated with an increase in the frequency.

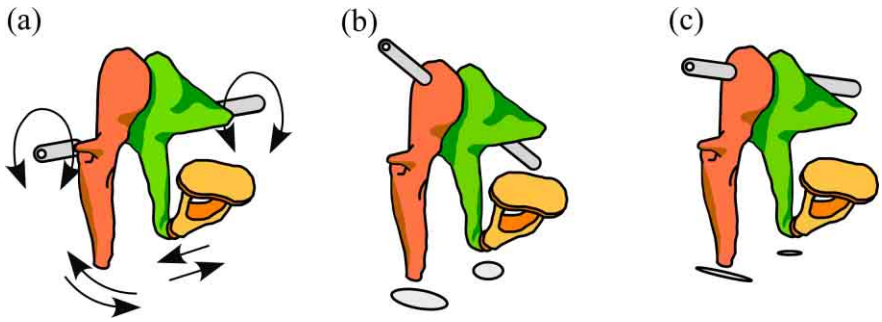

Figure 7. Rotational axis of the ossicular chain. Movements of the tip of the malleus handle and the stapes head are shown by arrows and ellipses. (a) Frequency f = 0.1 kHz. (b) f = 2.0 kHz. (c) f = 4.0 kHz. The rotational axis of the ossicular chain moves to the upper part of the ossicles with an increase in the frequency.

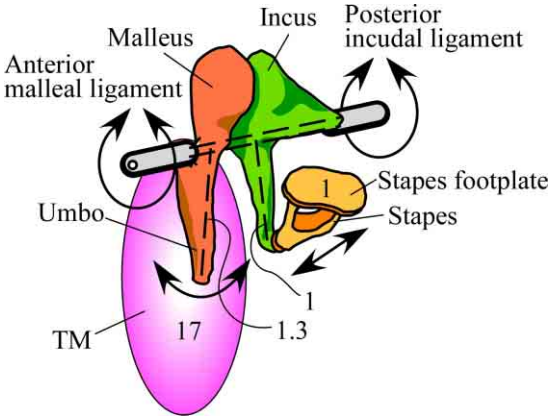

Figure 8. Dynamic behavior of the middle ear. Arrows indicate the directions of the movements. The ratio of the area of the tympanic membrane to that of the stapes is 17:1, and the ratio of the arm of the malleus to that of the incus is 1.3:1.0.

COCHLEAR FUNCTION: TRAVELING WAVES ALONG THE COCHLEAR PARTITION; THE MECHANO-RECEPTOR-TRANSDUCTION ROLE OF HAIR CELLS; RECENT DISCOVERY OF HAIR-CELL MOTILITY, AND THE "COCHLEAR AMPLIFIER"

Vibrations of the stapes generate movement of the cochlear fluids that interacts with the basilar membrane, the stiffness of which decreases from base to apex. This interaction produces progressive traveling waves on the basilar membrane [8], which are similar to waves beating upon a seashore. Figure 9 depicts these traveling waves [9]. When sound is transmitted to the basilar membrane, the position of the maximum

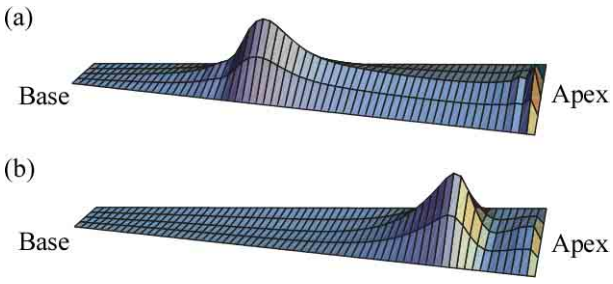

Figure 9. Traveling waves on the basilar membrane obtained from the finite element method model. (a) Input stimulus frequency f = 6.0 kHz. (b) f = 2.0 kHz. Traveling waves on the basilar membrane have a peak near the base when high frequency sound enters the cochlea, while low frequency sound develops the traveling waves on the basilar membrane, which have a peak near the apex.

displacement amplitude of its vibration is related to the frequency of the sound. In other words, each position along the basilar membrane has a maximum displacement amplitude at a specific frequency called the characteristic frequency. Figure 10 is a frequency map for humans showing characteristic frequencies at different positions in the ear [10]. As shown in Fig. 11, when sound enters the ear, the organ of Corti, which sits on the basilar membrane, undergoes a rocking motion. Although the details of cochlear operation are unclear, one possible mechanism is that basilar membrane displacement toward the scala vestibuli produces shear motion between the tectorial membrane and the reticular lamina and induces the flow of fluid in the direction of the arrow, which leads to the deflection of the free-standing inner hair cell stereocilia in the same direction as the flow [11, 12]. As shown in Fig. 12, this deflection induces the opening of ion channels and an influx of ions into the inner hair cell, thus releasing the transmitter. As a result, pulses are generated in the auditory nerve fibers, as shown in Fig. 13. Because of this mechanism, we can hear sound.

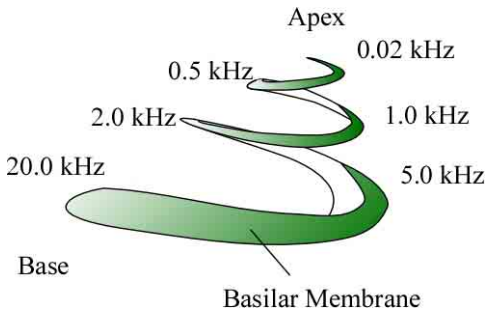

Figure 10. Place-characteristic frequency map for humans. High and low frequency components of sound are analyzed in the basal and apical regions of the cochlea, respectively.

As depicted in Fig. 14, the mammalian OHC is cylindrical-shaped with a radius of 4-5 μm and a length of 30-90 μm. Similar to the structure of the IHC, the OHC is capped by the cuticular plate with stereocilia at one end and by the synaptic membrane

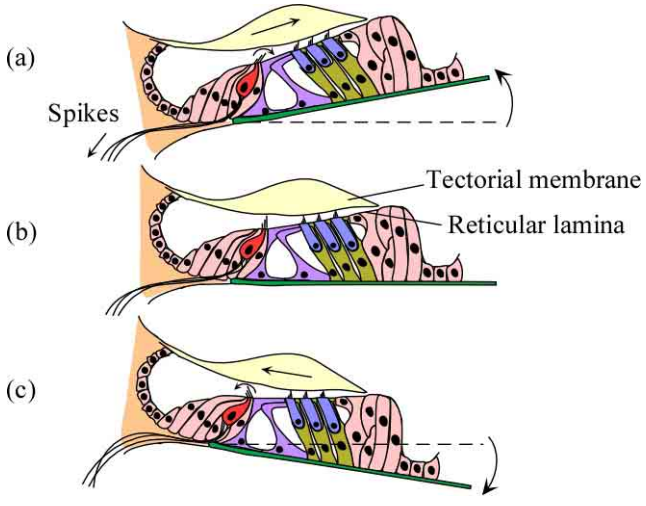

Figure 11. Vibration mode of the organ of Corti. (a) Displacement toward the scala vestibuli. (b) Resting position. (c) Displacement toward the scala tympani. The inner hair cell stereocilia are deflected by the flow of fluid caused by shear motion between the tectorial membrane and the reticular lamina.

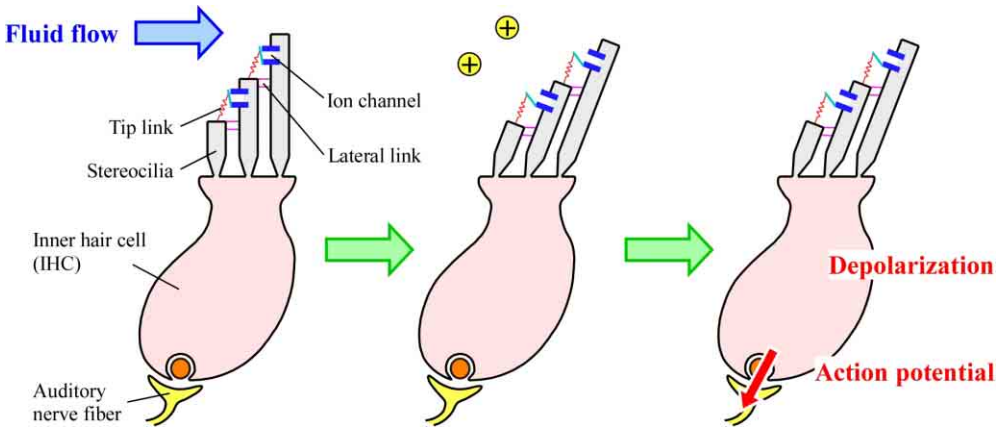

Figure 12. A bundle of stereocilia located on the apical surface of the IHC is deflected by the shear force caused by shear motion of the tectorial membrane against the reticular lamina. When the bundle is deflected in the excitatory direction, i.e., toward the tallest stereocilium, the tip link is under tension and the mechanoelectrical transduction channel located at the end of the tip link is thought to open [13]. Due to this, an influx of ions into the IHC is generated, which in turn depolarizes the membrane potential of the IHC and then produces action potentials in the auditory nerve fibers.

at the other end. When the stereocilia bend in the direction of the arrow, K^+ and Ca^{2+} ions flow into the cell and depolarize the membrane potentials, thereby resembling the function of the IHC. At the same time, the OHC contracts, whereas the IHC releases the transmitter and action potentials are produced in the auditory nerve fibers. By contrast, when the stereocilia bend in the direction opposite that of the arrow, the membrane potentials are hyperpolarized and the OHC elongates [14]. Figure 15 depicts an experiment where, instead of bending stereocilia, intracellular potentials are charged by the whole-cell voltage clamped technique. Experiments reveal that the input-output function of the OHC is not expressed by a straight line but a curved one, i.e., the function is non-linear, which is responsible for compressive nonlinear responses of the basilar membrane [15] and the cochlea [16]. Moreover, the OHCs are under efferent control [17].

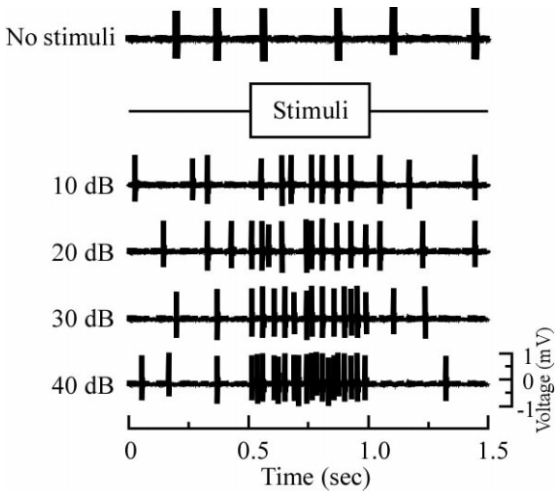

Figure 13. Response in the auditory nerve fiber. The upper waveform is an example of spontaneous activity. When a stimulus tone is delivered to the external auditory canal, the spike rate rises with an increase in the stimulus level.

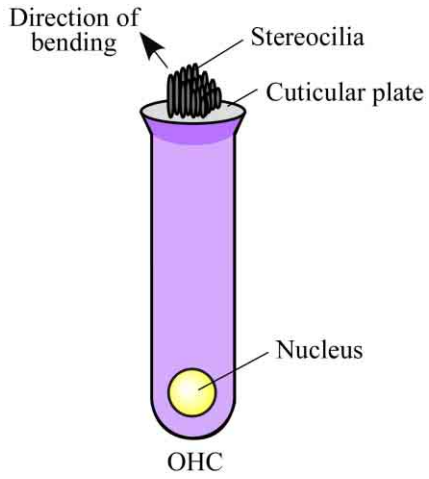

Figure 14. Schematic diagram of th outer hair cell. The polarity is th same as that of Fig. 11. The cell i capped by the cuticular plate wit stereocilia, which have a V o W-shaped formation.

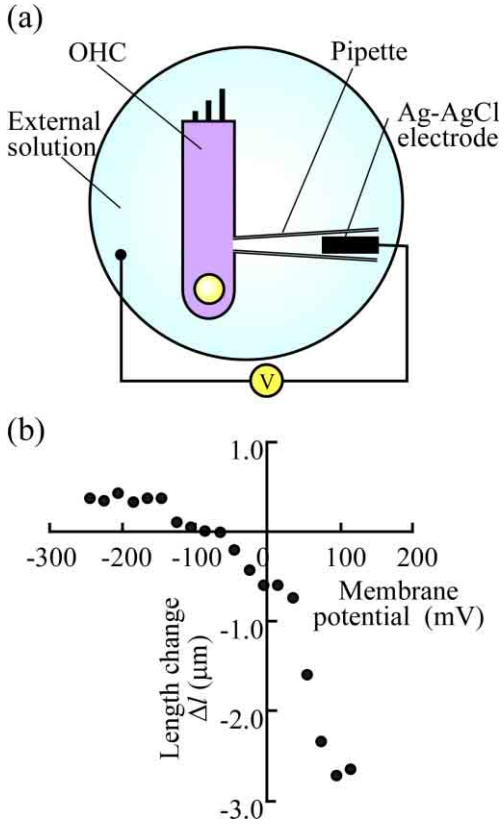

Figure 15. Measurement of outer hair cell motility. (a) Whole-cell voltage clamp technique. To elicit mechanical movements of the outer hair cells, step and sinusoidal voltage stimuli are given to the isolated outer hair cells. (b) Length change of the outer hair cell in response to step voltage stimuli. The positive and negative directions show cell elongation and contraction, respectively.

As shown in Fig. 11, when the organ of Corti is deflected toward the scala vestibuli, the OHC stereocilia bend due to the shear motion between the tectorial membrane and the reticular lamina, because the tallest OHC stereocilia adhere to the tectorial membrane. Simultaneously, the OHCs contract. Deflection of the organ of Corti toward the scale tympani leads to elongation of the OHCs. This repeated contraction and elongation of the OHCs, i.e., the motility of the OHCs, magnifies the deflection of the organ of Corti. To confirm this cochlear amplification, an attempt was made to directly measure the basilar membrane vibrations in both living and dead guinea pigs by a Laser Doppler Velocimeter [18]. Figure 16 clearly shows that amplification of the basilar membrane vibrations occurs when an animal is alive. The magnified deflection of the organ of Corti leads to increases in the movement of the fluids in the space near the stereocilia of the inner hair cells and in the deflection of the inner hair cell stereocilia. Owing to the mechanism mentioned above, our auditory system is characterized by high sensitivity, sharp tuning, and compressive nonlinearity [19-21].

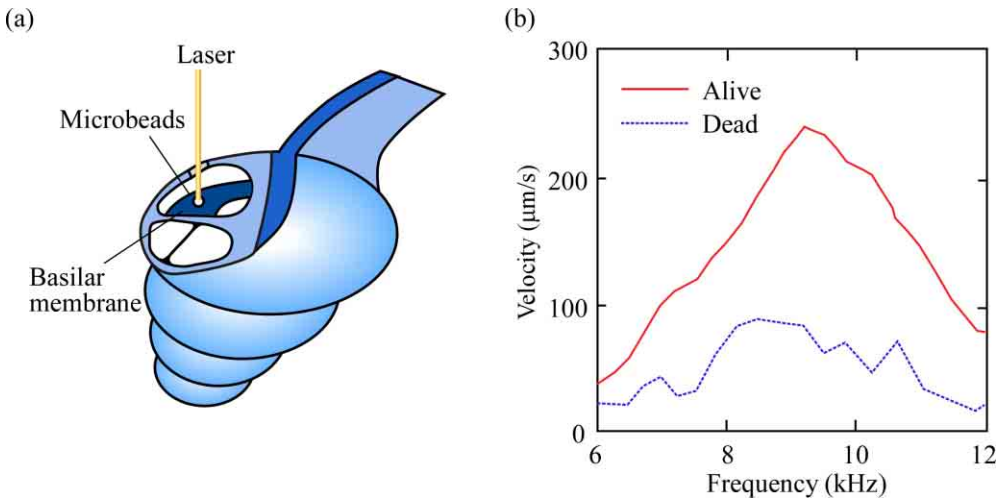

Figure 16. Direct measurement of basilar membrane vibrations in a guinea pig. (a) Measurement procedure. A hole with a diameter of 0.5 mm is opened at the bony wall of the cochlea, and glass microbeads with a diameter of 20 μm are placed on the basilar membrane in order to increase reflections of the laser beam. (b) The basilar membrane velocity responses to periodic tone. P = 75 dB SPL. The basilar membrane vibrations are amplified when an animal is alive.

THE ORIGIN OF THE MOTILITY OF OHCS, I.E., THE MOTOR PROTEIN "PRESTIN"

As shown in Fig. 17, motor proteins are thought to be embedded in the lateral plasma membrane of the OHCs. The source of the somatic length change of the OHCs is considered to be the conformational changes of these motor proteins. In 2000, the motor protein was identified in the gerbil cochlea and termed "prestin" [22]. The lateral wall of the OHC has been observed by electron microscopy. The existence of many particles, ten nanometers in diameter, in the plasma membrane has been shown using the freeze-fracture

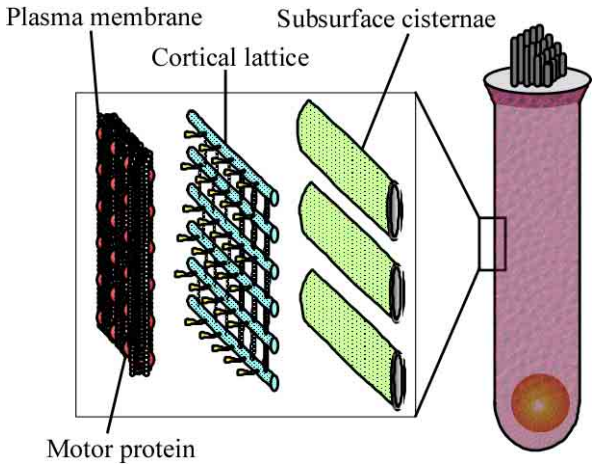

Figure 17. Lateral wall of the OHC. The OHC lateral wall has a unique trilaminate structure: the outermost plasma membrane, the cortical lattice, and the innermost subsurface cisternae. The motor protein is thought to be embedded in the plasma membrane.

technique [23-25]. These densely packed 10-nm particles in the lateral membrane of OHCs are thought to be motor proteins. However, it still remains unknown whether these particles are prestin or not.

The cytoplasmic surfaces of the isolated plasma membranes of the prestin-transfected Chinese hamster ovary (CHO) cells and those of the untransfected CHO cells were therefore observed by the tapping mode of AFM to visualize the membrane proteins [26, 26]. Figure 18 represents their original flattened AFM images and the calculated differential AFM images. Analysis of the shape and size of the observed structures was then performed in AFM images of the prestin-transfected CHO cells and those of the untransfected CHO cells. The frequency distribution of the observed particle-like structures, i.e., the density of the particle-like structures plotted against the interval of 2-nm classes in the diameter, is shown in Fig. 19. Since the difference between the prestin-transfected and untransfected CHO cells is due to the existence of prestin, the difference of the densities of the particle-like structures between the prestin-transfected CHO cells and the untransfected CHO cells is considered to be caused by the presence or absence of prestin. Based on Fig. 19, therefore, the density of prestin in the prestin-transfected CHO cells was estimated to

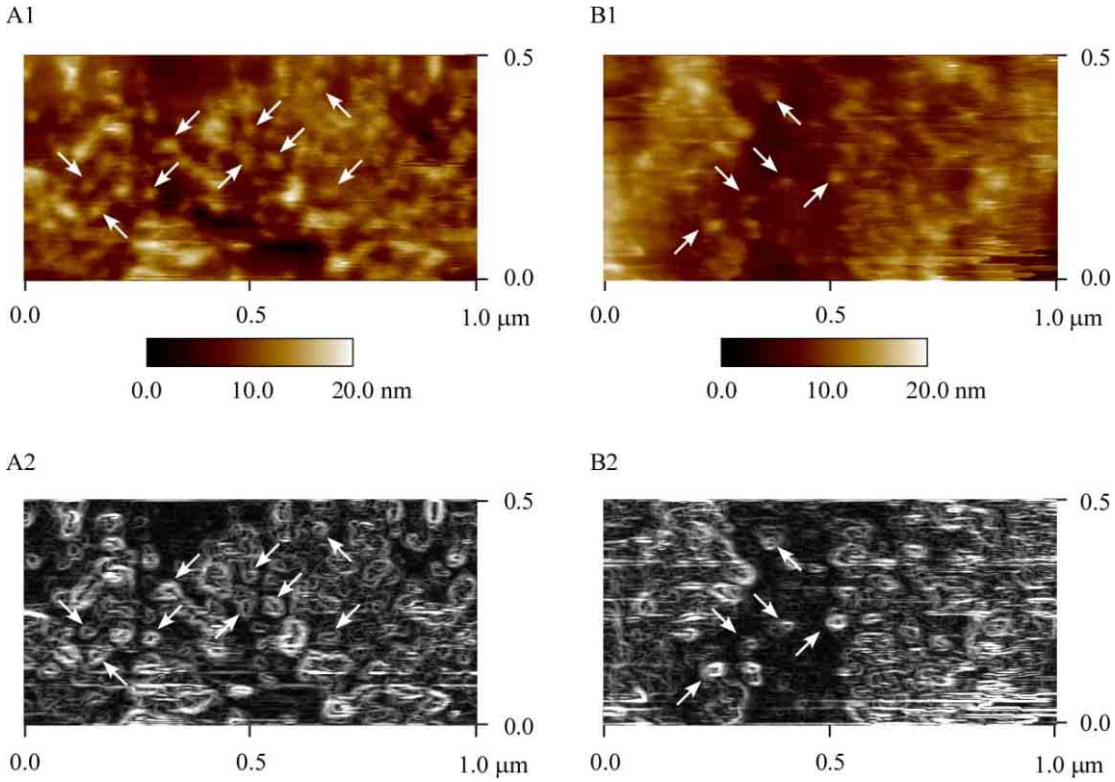

Figure 18. AFM images of membranes of the CHO cells. (A1) Original flattened AFM image of the prestin-transfected CHO cell. (A2) Differential AFM image of A1. (B1) Original flattened AFM image of the untransfected CHO cell. (B2) Differential AFM image of B1. Particle-like structures were recognized in the plasma membranes of the prestin-transfected CHO cells and the untransfected CHO cells, as indicated by arrows. No distinctive difference in such particle-like structures was found between these cells.

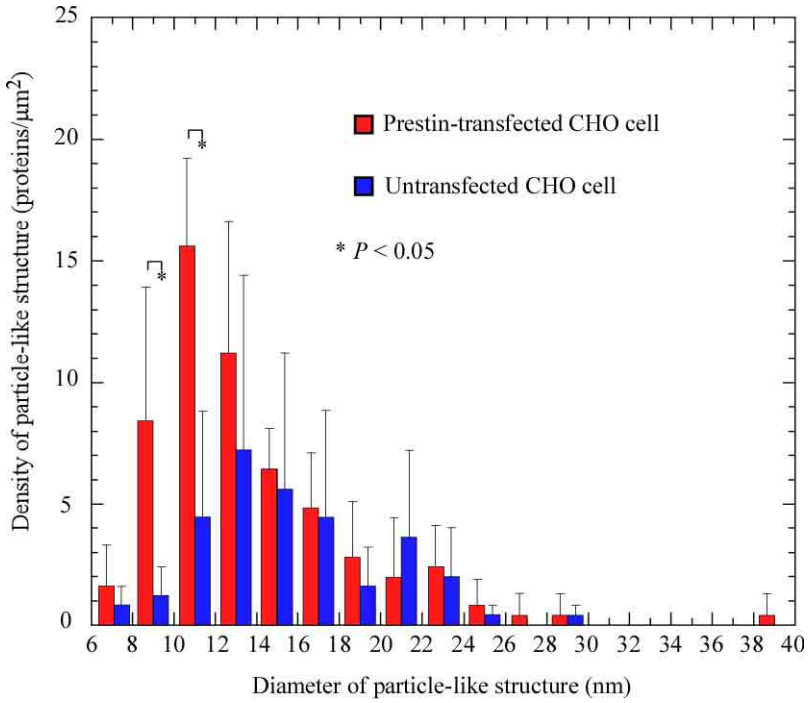

Figure 19. Frequency distribution of the observed particle-like structures in the plasma membrane. The density of the particle-like structures is plotted against the interval in 2-nm classes. Data were obtained from five AFM images of the prestin-transfected CHO cells and five such images of the untransfected CHO cells. When the sizes of the particle-like structures were 8–10 nm and 10–12 nm, differences of their densities between the prestin-transfected CHO cells and the untransfected CHO cells were statistically significant for $P < 0.05$ using Student's t-test, as shown by the asterisks. Error bars represent standard deviations.

be 18 ± 9 proteins/μm^2 (n = 5) after subtracting the value of the density of the particle-like structures in the untransfected CHO cells from those in the prestin-transfected CHO cells in the 8–12 nm class. This value corresponds to approximately 75% of the total density of the particle-like structures in the prestin-transfected CHO cell membrane. These results suggest that the majority of these particle-like structures with a diameter of 8–12 nm in the prestin-transfected CHO plasma membrane are possibly prestin.

CONCLUSIONS

An anatomical and functional overview of the human auditory system was herein presented, the main conclusions being as follows: High sensitivity and sharp tuning of our auditory system originate in the motility of the outer hair cells located in the organ of Corti in the cochlea, the source of this motility is the conformational changes of the motor protein prestin in the lateral wall of the cells, and the input-output function of the outer hair cell motility governs the non-linearity of our auditory system.

REFERENCES

[1] W. M. Robinson and R. S. Dadson, "A redetermination of the equal loudness relations for pure tones", Br. J. Appl. Phys., **7**, 166-181 (1956).

[2] R. L. Wegel, "Physical data and physiology of excitation of the auditory nerve", Ann. Otol. Rhinol. Laryngol., **41**, 740-779 (1932).

[3] L. Ulehlova, L. Voldrich and R. Janisch, "Correlative study of sensory cell density and cochlea length in humans", Hear. Res., **28**, 149-151 (1987).

[4] H. Wada, M. Andoh, M. Takeuchi, H. Sugawara, T. Koike, T. Kobayashi, K. Hozawa, T. Gemma and M. Nara, "Vibration measurement of the tympanic membrane of guinea pig temporal bones using time-averaged speckle pattern interferometry", J. Acoust. Soc. Am., **111**, 2189-2199 (2002).

[5] T. Koike, H. Wada, T. Kobayashi, K. Ohyama and T. Takasaka, "Theoretical analysis of sound transmission in human middle ear and its effects on otoacoustic emissions". *In: H. Wada et al. (eds.), Recent Developments in Auditory Mechanics, 71-77* (World Scientific Publishing, Singapore, 2000).

[6] W. F. Decraemer and S. M. Khanna, "New insights into vibration of the middle ear". *In: J. J. Rosowski and S. N. Merchant (eds.), The Function and Mechanics of Normal, Diseased and Reconstructed Middle Ears, 23-38* (Kluwer, The Hague, 2000).

[7] T. Koike, H. Wada and T. Kobayashi, "Modeling of the human middle ear using the finite-element method", J. Acoust. Soc. Am., **111**, 1306-1317 (2002).

[8] G. V. Békésy, *Experiments in Hearing*. (McGraw-Hill Book Company, Inc., New York, 452-455, 1960).

[9] Wada Laboratory, Dynamic animation of the basilar membrane, Available at: http://www.wadalab.mech.tohoku.ac.jp/.

[10] G. V. Békésy, *Experiments in Hearing*. (McGraw-Hill Book Company, Inc., New York, 441-443, 1960).

[11] H. Wada, A. Takeda and T. Kawase, "Timing of neural excitation in relation to basilar membrane motion in the basal region of the guinea pig cochlea during the presentation of low-frequency acoustic stimulation", Hear. Res., **165**, 165-176 (2002).

[12] M. Andoh, C. Nakajima and H. Wada, "Phase of neural excitation relative to basilar membrane motion in the organ of Corti: Theoretical considerations", J. Acoust. Soc. Am., **118**, 1554-1565 (2005).

[13] J. O. Pickles, S. D. Comis and M .P. Osborne, "Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction", Hear. Res., **15**, 103–112 (1984).

[14] W. E. Brownell, C. R. Bader, D. Bertrand, Y. de Ribaupierre, “Evoked mechanical responses of isolated cochlear outer hair cells”, Science, **227**, 194-196 (1985).

[15] L. Robles, M. A. Ruggero and N. C. Rich, “Basilar membrane mechanics at the base of the chinchilla cochlea”, J. Acoust. Soc. Am., **80**, 1364-1374 (1986).

[16] A. Cohen and M. Furst, “Integration of outer hair cell activity in a one-dimensional cochlear model”, J. Acoust. Soc. Am., **115**, 2185-2192 (2004).

[17] E. Murugasu and I. J. Russell, “The effect of efferent stimulation on basilar membrane displacement in the basal turn of the guinea pig cochlea”, J. Neurosci., **16**, 1306-1317 (1996).

[18] H. Wada, Y. Honnma, S. Takahashi, T. Takasaka and K. Ohyama, “Simultaneous measurement of DPOAEs and basilar membrane vibration by acoustic probe and laser Doppler velocimeter”. *In: E. R. Lewis et al. (eds.), Diversity in Auditory Mechanics, 284-290* (World Scientific Publishing, Singapore, 1997).

[19] J. O. Pickles, *An Introduction to the Physiology of Hearing*. (Academic Press, London, 136-157, 1988).

[20] C. D. Geisler, *From Sound to Synapse*. (Oxford University Press, New York, 125-138, 1998).

[21] J. L. Goldstein, “Auditory nonlinearity”, J. Acoust. Soc. Am., **41**, 676-689 (1867).

[22] J. Zheng, W. Shen, D. Z. Z. He, K. B. Long, L. D. Madison and P. Dallos, “Prestin is the motor protein of cochlear outer hair cells”, Nature, **405**, 149–155 (2000).

[23] T. Arima, A. Kuraoka, R. Toriya, Y. Shibata and T. Uemura, “Quick-freeze, deep-etch visualization of the ‘cytoskeletal spring’ of cochlear outer hair cells”, Cell Tissue Res., **263**, 91–97 (1991).

[24] A. Forge, “Structural features of the lateral walls in mammalian cochlear outer hair cells”, Cell Tissue Res., **265**, 473–483 (1991).

[25] F. Kalinec, M. C. Holley, K. H. Iwasa, D. J. Lim and B. Kachar, “A membrane-based force generation mechanism in auditory sensory cells”, Proc. Natl. Acad. Sci. USA, **89**, 8671–8675 (1992).

[26] K. Iida, K. Tsumoto, K. Ikeda, I. Kumagai, T. Kobayashi and H. Wada, “Construction of an expression system for the motor protein prestin in Chinese hamster ovary cells”, Hear. Res., **205**, 262-270 (2005).

[27] M. Murakoshi, T. Gomi, K. Iida, S. Kumano, K. Tsumoto, I. Kumagai, K. Ikeda, T. Kobayashi and H. Wada, “Imaging by atomic force microscopy of the plasma membrane of prestin-transfected Chinese hamster ovary cells”, JARO, in press.