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PART IV: ANIMAL RESEARCH IN PERIPHERAL AUDITORY PHYSIOLOGY

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BACKGROUND

Cataloging the microanatomy of the auditory end organ provided an important start for understanding the physiology of the peripheral auditory system. These anatomical studies, conducted on a variety of species, began in earnest following the development of the compound light microscope in the 1700s. The detail in some of the original work of such pioneers as Alfonso Corti is remarkable (see Hawkins[1] and references within), but alas the microscopic structures were necessarily altered by the decay following extirpation and dissection. As we shall see, this decay had a major impact on our understanding of cochlear physiology as well. Whereas the development of fixatives proved a boon for anatomists, physiologists would not benefit directly but would belatedly profit from histologists' appreciation of the need to preserve the natural state.

The cochlea, we now know, is a remarkable electromechanical device. Prior to the discovery of animal electricity and means to measure it, only the mechanical components of cochlea function could be investigated. Thus, the microscopic structures in the inner ear were used to deduce the mechanical foundation of cochlea function; for example, Helmholtz postulated his resonance theory based on the changing length (hence mechanical properties) of radial basilar membrane fibers. Clearly, in mammals, the basilar membrane with its varying dimensions and stiffness would be a prime candidate for the basis of hearing. Indeed, von Bekesy's studies[2] confirmed the importance of the basilar membrane's mechanical qualities by demonstrating tuned traveling waves evoked by basilar membrane disturbances. von Bekesy used a variety of animal models, mainly the human temporal bone, but also mouse to elephant, to assess the sharpness of tuning in the cochlea, and while the data he obtained contributed immensely to our understanding of cochlea mechanics and won him the Nobel prize in 1961, unforeseen factors worked to stymie an understanding of the true sensitivity and tuning of the mammalian cochlea. These factors were the same that stymied early anatomists, namely the difficult-to-avoid decay of normal cellular structure and function after death. These problems, though acknowledged by von Bekesy, were argued away, so that for many years auditory aficionados were led to believe that his data were gold. In fact, there were serious problems with his experiments, which when ultimately realized and solved would help elucidate the roles of the two types of hair cells, the inner and outer hair cells, that the early anatomists identified with the aid of improved fixation techniques. To improve on the work of von Bekesy, models other than the human would be required.

WHY DO WE STUDY ANIMAL MODELS?

There are two main reasons for the study of peripheral auditory physiology, namely, the quest to understand and cure auditory dysfunction, and simply to understand how our and other animals'

ears work. Clearly, study based on the former rationale would benefit from direct investigations on humans. Unfortunately, though, immense difficulties are associated with human experimentation on the auditory periphery. These problems include limited number of specimens, inability to control for pathological effects, and the inevitable problem of tissue destruction caused by delays in the access to the cochlea (caused by dissection difficulties due to the dense temporal bone and delays in acquisition following death). Thus, for many reasons and regardless of the clinical vs. scientific rationales, animal models are best for studies on cochlear physiology. The major hurdle in rationalizing a particular animal model is its validity and generality. As far as providing insight to the human condition, most investigators believe that a range of small mammals, including cat, chinchilla, guinea pig, rat, and mouse, are appropriate. Of course, one or another species may prove better for particular experimental questions. In general, however, these small animals are particularly well suited since very nice access to the cochlea structures can be had. This enhanced accessibility (and heightened concern for better physiological status) has led directly to enhancements of the findings of von Bekesy.

BASILAR MEMBRANE TUNING

The data on basilar membrane movement that von Bekesy obtained indicated that the response of the basilar membrane was linear, i.e., the magnitude of the displacement response grew linearly with the stimulus level. By extrapolating down to threshold levels, it was argued that basilar membrane movements at the threshold of hearing were a fraction of an atom's width. Tuning of the basilar membrane was also observed to be not as good as psychophysical measures, and von Bekesy sought reasons beyond the basilar membrane to explain how we hear so well. With the aid of animal models, these notions would dramatically change.

Using the squirrel monkey, Rhode and colleagues[3,4] first noticed that in some animals a nonlinear growth in basilar membrane motions occurred as stimulation levels were changed. Importantly, this compressive nonlinearity of the basilar membrane response was vulnerable to the animal's status. Only when extreme care was used to maintain a healthy preparation did the response remain nonlinear, otherwise it resembled the data of von Bekesy. We know now that tuning on the basilar membrane is far sharper than the data of von Bekesy showed, indicating that his human temporal bone preparation and his animal preparations were working in a passive, damaged mode. Of course, the new Mossbauer measurement tool that Rhode and colleagues used helped to record at lower stimulus levels than von Bekesy could using stroboscopic illumination. Today, it is clear that the sharp tuning found in the eighth nerve has its direct counterpart in basilar membrane motion.[5] This turnaround in the description of basilar membrane tuning highlights the caveat that while animal models have the potential to provide valid data, care must be exercised in order to attain validity.

THE COCHLEA IS ELECTRIC

In the 1700s, electricians (as serious students of electricity were then called) often relied on themselves or acquaintances to serve as subjects. Indeed, Volta perceived a deafening blast as a result of self-inflicted aural electrocution.[6] Despite this early indication, it would be nearly two centuries after the controversial discovery of animal electricity that the electrical nature of audition would be confirmed. The discovery had to wait for the invention of the vacuum tube and oscilloscope, but once in hand Wever and Bray[7] demonstrated the "cochlear microphonic," an electrical response of the hair cells (which they mistakenly took as eighth nerve firings) measured from electrodes placed on a decerebrate cat's eighth nerve. The response mimicked the acoustic stimulus, and they confirmed this finding in a number of animal species, including turtles and insects.

The study of the electrical activity of the cochlea took off, and small mammals were perfect models since their cochleae were easily exposed for electrode implantations. Extracellular (from scala media and the perilymphatic scalae) electrical recordings were made of the resting (e.g., endolymphatic potential) and sound-evoked potentials (cochlea microphonic, summating potential, and even action potentials; see Dallos[8]), but eventually these were usurped by intracellular recording directly from hair cells[9,10] after the high impedance electrode was devised. The clear correspondence of electrical activity within the auditory periphery with hearing capabilities would place this measure above that of histopathologic determination of auditory insult and recovery.

ANIMAL MODELS OF CLINICAL IMPORTANCE

The anatomical identification and characterization of cochlear dysfunction is time-consuming. The classic histological work on otopathology (see Schuknecht[11]), while quite informative, could not be used with high efficiency, simply because the animal or human temporal bone had to be removed and processed after death. The use of electrical measures to determine auditory sensitivity, especially those that were noninvasive (e.g., auditory brainstem response [ABR], compound action potential [CAP]) permitted ongoing and quantitative studies on the effects of noise exposure, ototoxic drug exposure, and hazardous chemicals (e.g., see Henderson and coworkers[12]). As a direct result of animal experimentation, we would no longer have to experience devastation such as that caused by the first uses of the ototoxic antibiotic dihydrostreptomycin.[13] Nevertheless, while animal models have immensely contributed towards our understanding of pathologies that afflict humans, we must remain cognizant of species-specific differences.

One of the hottest areas of current research is that of hair cell regeneration (see Warchol[14]). Mammalian hair cells do not regenerate following destruction, as do those of some lower vertebrates. Thus, unlike some tissues that can be interrogated via cell culture, mammalian inner ears must be harvested for each new experiment. Perhaps some day, if the key that controls hair cell production is found, hair cell cultures may reduce the need for animal sacrifice.

In the heyday of electron microscopy, transport of electron-dense markers into various compartments of the inner ear was studied. These studies included, for example, movements of molecules out of the vasculature and into cochlea scalae, thus identifying the blood–labyrinthine barriers[15–17] and movements of molecules across the round window membrane into the scala tympani.[18] The latter experiments contributed to the scientific basis for the currently popular clinical approach of intratympanic drug delivery, notably used to deliver gentamycin for control of intolerable vertigo. This type of approach heralds a new era where the otologist will successfully treat previously inaccessible structures of the inner ear and will ultimately do so with the new tools of molecular biology.

FROM MOLECULES TO EAR

Some very important advances in auditory physiology have been made in recent years using the techniques of molecular biology. Two of them are especially dear to my heart, namely, the determination that mutations of the connexon 26 gene result in nonsyndromic deafness[19] and the identification of the protein responsible for the mechanical activity of the outer hair cell.[20]

Connexons are proteins that form gap junction channels between adjacent cells, in the ear's case, allowing ionic and metabolic communication between supporting cells.[21] These channels were proposed to aid in the removal of harmful extracellular potassium away from active hair cells,[22] and this is a likely reason for the mutation's devastation of hearing.

The mammalian outer hair cell has long been known to dance wildly in response to electrical stimulation;[23–25] this mechanical activity is believed to promote the sharp tuning and nonlinearity

that Rhode and colleagues found in the basilar membrane. Finally, after nearly two decades, the motor responsible for the outer hair cell (OHC) boogie was molecularly identified as the protein prestin.[20] The story is a continuing one that is full of twists,[26] but most recently a knockout of the prestin gene in the mouse seriously interfered with normal auditory function,[27] and a mutation of the gene was shown to cause deafness in humans.[28] In the end, it will likely be the mouse model that holds the key to our interests in the ear, as this small prolific rodent is a perfect molecular biology laboratory. But let us not forget the guinea pig, which is a classic model in auditory research; indeed, this animal has just helped show that gene transfection (of Math1 transcription factor) into the intact cochlea can induce new hair cell growth.[29] Imagine, the future otologist growing some new hair cells for us hard-of-hearing baby boomers — thanks to animal research.

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