The neuroscience of hearing or how to do a hard job with soft components

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Abstract
The inner ear is a small and relatively inaccessible structure. The use of multiple biophysical recording techniques from the late 1970s onwards, combined with molecular genetics to identify genes critically involved in cochlear development, has revealed how the cochlea acts as the front end for the central nervous system analysis of the auditory world. Some notable progress has been made in clarifying the mechanisms of frequency coding and cochlear amplification, and of mechano-transduction in hair cells and in establishing molecules necessary for normal (and by implication in abnormal) development of hearing and balance. There has been a parallel growth in understanding some of the neural networks in the brainstem and cortical areas responsible for processing the information derived from the auditory nerve. Informing future technical improvements to hearing aids and cochlear implants (electrically and optogenetically encoded), this chapter concentrates mainly on the neuroscience of peripheral hearing.

A little history

When the BNA (or the Brain Research Association as it was then) first held its meetings, there was remarkably little known about how the ear worked. How the cochlea performed was considered to be an engineering project: the major laboratories were those clustered around MIT and the Bell Labs, mainly as offshoots of signal processing and communications groups. It was known that individual auditory nerve fibres were sharply tuned to specific frequencies, often showing high spontaneous firing rates and which could be stimulated to fire transiently at rates approaching 1000 Hz. Few groups were equipped to study the system: the equipment was complex and benefitted from the first neural processing computers based in those US laboratories. In the United Kingdom, the group around Ted Evans at the Department of Communication and Neuroscience at Keele (a department set up by Donald Mackay in 1963 which only added the 'Neuroscience' label in 1973) became one of the first to produce definitive extracellular recordings documenting the auditory nerve firing patterns.

Even though Georg von Bekesy had been awarded a Nobel Prize in 1961 for his work on cochlear mechanics, there was a serious mismatch between the pattern of the basilar membrane vibration and the tuning of individual fibres. The underlying cell physiology in the cochlea was unknown. It remained an inaccessible structure for physiological research. In line with engineering ways of thinking about the problem, the sharp neural tuning was ascribed to a 'second filter', serving to select only limited frequencies to provide the drive for neural firing.

The high ground for understanding the cell biology of the cochlea was held by Sweden, with several groups, mainly in Stockholm, carrying out the first electron microscopy and even recording very small receptor potentials from sensory hair cells in *Necturus* when the bundle was deflected (Harris et al., 1970). By 1980, however, the field had begun to open up even though the number of groups still remained small, certainly compared to the effort that was being devoted to the visual system. Jim Hudspeth, who had spent time as a visiting fellow in Ake Flock's lab at the Karolinska, showed that hair cells from the frog could be reliably stimulated and recorded in vitro (Hudspeth and Corey, 1977). Ian Russell and Peter Sellick at Sussex started to record from hair cells in the in vivo guinea pig cochlea, showing that the cells were already sharply tuned. In completely different developments, Andrew Crawford and Robert Fettiplace in Cambridge showed that hair cells from the turtle were tuned, but using mechanisms intrinsic to the cells. What made these advances were technical improvements in recording from cells with fine microelectrodes, techniques which had been pioneered to study the neural networks of the retina.

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1980s

Four other discoveries changed the landscape of hearing research. First, David Kemp (1978) in London reported, to universal incredulity, that the ear emitted sound. This had been predicted by Thomas Gold in the late 1940s, but the microphone sensitivity was not sufficient to show it at the time. Second, a number of groups revisited von Bekesy's experiments and showed that the basilar membrane, the frequency defining feature of the mammalian cochlea was, after all, reliably tuned, provided that the animal was in good physiological condition and not dead, as was the case in the original experiments (Sellick et al., 1982). Third, Jim Pickles in Birmingham spotted that many of the electron micrograph images of the hair bundle exhibited a very fine link – termed a 'tip-link' – running between adjacent stereocilia (the 'hairs') and that this provided an explanation for why the bundle could only be excited when pushed in one direction. It incidentally hinted that the transduction of movement in vertebrate hair cells was associated with a molecular complex at the tips of the hair cells. This precise nature of this complex remains unresolved.

The outer hair cells of the cochlea, cells three times more numerous than the inner hair cells, had an indeterminate role. From the 1970s it was realised that sharp tuning in mammalian auditory nerve fibres was severely reduced when this population was selectively destroyed by drugs or noise. The cells even appeared to be motile and changed length when electrically stimulated (Brownell et al., 1985). Flock and his coworkers had shown earlier that the stereocilia contained actin and had suggested that outer hair cells could be part of a motor control system rather like the spindles operating in muscle. Outer hair cell motility was something different however. It took a high bandwidth recording and stimulation afforded by patch clamp technologies to show that outer hair cells could generate forces fast enough to be involved in the modulation of the basilar membrane motion required for acoustic frequencies (reviewed in Ashmore (2008)).

1990s

The cochlea is a small structure buried within the temporal bone so that unlike the retina, where there is much more tissue to use, molecular biological techniques arrived a little later. However, by the 1990s, molecular techniques started to identify many of the genes involved in hearing. An early result from 1989 by Allen Ryan in San Diego identified a critical transcription factor POUF43 in inner ear development, but the identification of mutations in PAX3, and MYO7a as genes leading to deafness and the mouse and humans alike really started the activity (Gibson et al., 1995). Following this, many genes key to cochlear and vestibular development have been identified. An intelligent guess at the beginning of the decade that perhaps 10–20 genes would be critical to cochlear development has given way to the realisation that there are now more than 200 genes (and counting) and many more loci all involved in the normal physiology of the cochlea. Of particular interest has been the identification of mutations in the gap junction protein Cx26 expressed in the membranes of the cochlear supporting cells. Mutations in its gene account for approximately 40% of hereditary hearing loss in the human population. Interest also focussed on genes associated with Usher syndrome, a condition which can lead to blindness and hearing loss (Richardson et al., 2011). The Usher syndrome genes encode proteins which are associated with the construction and functioning of the hair bundle, the defining organelle of the hair cell.

2000s and beyond

The molecule responsible for motility in cochlear outer cells was identified in Zheng et al. (2000). Termed *prestin*, as its presence makes hair cells move quickly, it is a single protein located down the side of the outer cell membrane. Surprisingly, it is a member of a family of nominally chloride-bicarbonate exchange transporters. Other member of this family, SLC26, is found in many tissues, and it is now thought that the prestin protein has evolved in mammals to lose many of its transport abilities. The precise structure of the protein remains currently unknown although more recently the homologous membrane protein, dgSL26, from the bacterium *Deinococcus geothermalis*, has been identified and is found to be an obligate dimer (Geertsma et al., 2015). Coupled with evidence that mammalian prestin is a tetramer (Hallworth and Nichols, 2012), there may be progress towards a better molecular description of this ultrafast actuator protein.

The past two decades have also made considerable progress in beginning to understand the sequence of events required to build the complex structure of the adult cochlea. Much of this has been possible by the development of organotypic cultures, systems of hair cells and their surrounding cells removed at embryonic stages and allowed to develop in vitro and by the extensive use of the mouse as a model of mammalian hearing.

Hair cells are not just mechanoreceptive cells but are presynaptic to the auditory nerve. The synapse of the inner hair cells is formed by 10–20 ribbon synapses, similar to those in photoreceptors, but specialised for the rapid vesicular release of glutamate. The triggering mechanism appears to be subtly different from that found in neuronal synapses: the normal array of synaptogamins is not present and vesicle cycling appears to place high demands on the supply of neurotransmitter vesicles. One possible specialisation for this synapse may be that there is a multifunctional protein otoferlin which doubles as calcium sensor and vesicle fusion protein (Michalski et al., 2017). There have been some very elegant studies focussing on the microdomains of calcium channels clustered around the ribbon which ensure that the release preserves high temporal fidelity.

Where do we go from here? Other auditory structures

The auditory neuroscience community of necessity is a combination of neuroscientists involved in every aspect from molecular mechanisms, through developmental cell biology and cell biophysics, neural computation, to brain imaging and psychoacoustics. Although the field is relatively well integrated internationally, it is still quite small compared, say, to that devoted to vision.

Molecular biology techniques have produced discovery techniques for key components of the hearing chain but have not resolved all the problems. An outstanding missing link in understanding hearing mechanisms is the identity of the mechano-electric transduction channel. There have been several false starts. It is certainly part of a complex of several proteins, not all

identified whose assay is its correct biophysical role in the hair cell. Nobody has yet devised a convincing expression system for the complex.

The best candidate for the transduction channel is a heteromer of TMC1 and TMC2 (Pan et al., 2013), but any additional ancillary components are unclear. Curiously, the mouse ('Beethoven') carrying a mutation in TMC1 (and as a result, deaf) was one of the first to be characterised genetically although at the time it was not identified specifically with their cell transduction.

The main issue at the moment is how the channel proteins link to other known proteins of the transduction complex. When sound deflects the hair bundle on the apical surface of the hair cell, the mechanical force is transmitted to the complex by a 'tip link', a protein chain consisting of cadherin23 and protocadherin15 (reviewed in Corey et al. (2017)). The link to the channel seems to be formed by at least two further proteins LHFPL5, the binding partner to the tip link, and TMIE, a channel accessory protein. The final assembly remains unknown.

The other end of the hair cell has also received considerable interest as the ribbon synapse is relatively accessible and is an excellent model for fast release synapses. The first auditory synapse plays an important role in thinking about hearing. More recent data show that after loud sound exposure, potentially a damaging stimulus, the auditory nerve reorganises itself. High-sensitivity, low-threshold fibres are retained (Kujawa and Liberman, 2015). What is lost, however, are the fibres which respond to high level sounds. The underlying cellular re-organisation is not understood. As an underlying planar cell polarity problem, how the hair cell organises itself into a structure so that it is excited preferentially in one direction remains a neurobiology problem for the future.

Outstanding problems

Hair cell regeneration

The development of the cochlea is complete by about week 26 in humans. In rodents much of the development occurs during the first postnatal 2 weeks and so is experimentally accessible. Although in non-mammalian species (birds for example) hair cells do have a capability for regeneration, as far as we know this genetic programme in mammals has been altered and the sensory cells do not regenerate. A considerable effort is being devoted to the problem. Some limited conversion of the supporting cells into hair cells has been reported, perhaps more successfully in the vestibular system, but the degree of hair cell replacement is quite minimal. More promising has been the use of stem cells (Chen et al., 2012). The problem of delivery of the cells into the adult cochlea and at the right place remains an outstanding technical problem.

Gene therapies

Perhaps more promising are the early signs that cochlear genes can be manipulated. A number of viral gene delivery systems have been shown in mouse models to be able to deliver genes to correct gene defects. These have been reported only for gene delivery to early stage mouse cochleas before the auditory system has developed fully; the prospects for altering the adult cochlea are unclear. So far, the most successful results – particular to correct defects in Usher genes and in one of the genes implicated in transduction genes – are based on adenovirus vectors, but the size of the gene cargo is limited to about 5 k bases at present. The ability to deliver larger genes into the inner ear is required as many of the critical genes overtly expressed in the cochlea are definitely larger. There is little doubt that the considerable effort will be devoted to better technologies for gene delivery, and with growing knowledge of the gene programmes, a degree of tailored manipulation of the cells of the inner ear may be possible.

The bioengineering of hearing aids and cochlear prostheses

Hearing has been the sense where bioengineering solutions to deficits have been with us for a long time (just think of hearing trumpets). The small digital hearing aids now available are really examples of the growth of consumer electronics, and the sophisticated devices now available, on the NHS for example, are sensibly priced and have almost reached the status of self-prescription that reading glasses have achieved. Hearing aids are still for the most part simple amplifying devices with relatively simple dynamic adaptations to different listening conditions. There are many opportunities to improve the way in which such devices intelligently interact with the cognitive state of the individual and indeed the other devices we now find around us in our daily lives.

The most impressive prosthesis used to improve hearing is the cochlear implant, where the auditory nerve is stimulated directly by an array of up to 26 electrodes inserted into the cochlear spiral. The central nervous system can use the limited number of channels possible in such a system, and there are clearly 'star' users among the current nearly 400,000 implants worldwide for whom 'hearing' is functionally effectively restored. The future developments for improving the implant stimulation include optogenetic methods to achieve much more selective stimulation of individual nerve fibres (Moser, 2015). The current limitation of such technologies is the relatively slow kinetics of the light activated ion channels once they are inserted into the nerve. Other optical stimulation techniques at different wavelengths offer future technological avenues. Stimulation does not have to be restricted to the cochlear nerve and other approaches using direct activation of subsets of the central auditory pathways are promising.

Hearing and cognition

Helen Keller is quoted as saying 'Blindness separates people from things; deafness separates people from people'. There is some evidence that there is a connection between hearing loss and the early signs of dementia (Lin et al., 2011), and although it might be thought that the social isolation resulting from hearing loss is a causative link, the evidence so far is quite anecdotal. Considerably more work needs to be carried out, but this remains an important area where the links, at the moment in their infancy, between central neural networks of hearing, their plasticity and their modulation by attention, can be developed.

Declaration of conflicting interests

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