NEUROBIOLOGY

Auditory fidelity

Thomas D. Parsons

Detailed investigation of a molecule involved in an inherited type of deafness reveals a fresh facet to the mammalian auditory system — a hitherto unknown way for synapses to put calcium in a bind.

Mammals react to sounds with exquisite temporal fidelity, a feat that is initiated by precise calcium-dependent signalling in the hair cells of the inner ear. Calcium is a common signalling molecule in the nervous system and elsewhere. Writing in *Cell*, Roux *et al.*[1] describe how they have identified an unexpected player in the auditory system. That player is a molecule called otoferlin, which has not previously been implicated as a calcium sensor for neurotransmitter release in nerve function.

A sensitive auditory system confers a tremendous evolutionary advantage, as it protects us from the things we fear most — those we cannot see. The ability to localize these potential dangers or communicate these possible threats depends on the precise timing of signals in the neural code that the brain ultimately perceives as sound. The basis of this temporal fidelity lies in the control of communication between the mechanosensory cell of the cochlea, the inner hair cell, and its downstream partner, the auditory nerve (Fig. 1). The mechanical energy of acoustic waves causes minute displacements of sensory hair bundles extending from the cell. These deflections result in rapidly oscillating electrical potentials, which trigger calcium influx from outside the cell; that in turn prompts tiny subcellular organelles filled with a chemical messenger to dump their contents into a well-defined extracellular compartment, the synaptic cleft. This messenger, or neurotransmitter, excites a nearby, afferent nerve fibre, which elicits an all-or-none electrical response that propagates details of the acoustic stimulus to the brain.

The capacity of the auditory system to follow acoustic waves oscillating at several thousand times per second suggests that this calcium-dependent regulatory event may need to be as much as ten times more precise than signalling between most types of neuron. So how does the inner hair cell achieve such exquisite temporal fidelity in its release of neurotransmitter? Roux *et al.*[1] suggest that the hair cell has evolved a unique calcium-sensing molecule, otoferlin, for controlling neurotransmitter release. The action of otoferlin allows a hair cell's specialized synapses — ribbon synapses, a specific class of afferent synapse common to sensory systems — to meet the requirements of hearing.

Both mice and humans suffer from an inherited form of deafness called DFNB9. Defects in otoferlin are responsible, and Roux and colleagues hypothesized that otoferlin might be involved in the correct operation of the synapse between the hair cell and the afferent nerve fibre. They found that, in mice, not only is otoferlin localized to the synaptic vesicles of inner hair cells, but that it also undergoes developmental changes in expression concurrent with the formation of ribbon synapses. Otoferlin also binds in a calcium-dependent manner to SNARE proteins, highly conserved molecules thought to be essential for the release of neurotransmitters and for other events requiring fusion of membranes.

The authors genetically manipulated the otoferlin molecule to prevent its functional expression. Mice lacking both copies of the normal gene have structurally normal synapses between the hair cell and afferent fibre, but are deaf and lack calcium-triggered dumping of the synaptic-vesicle contents. Interestingly, only the most rapid phase of putative neurotransmitter release is abolished by a defect in the otoferlin molecule. This fast component is widely thought to be associated with a specialized class of the vesicles that are close to the cell surface and molecularly poised for release (Fig. 1).

This paper[1] is of great interest to both specialists in hearing research and neuroscientists in general, for several reasons. First, the mystery of the molecular entity mediating the temporal fidelity of signalling by the primary synapse in the auditory system may now be solved. At most fast synapses, the transmembrane protein synaptotagmin I is thought to be the calcium sensor of fast, synchronized neurotransmitter release[2,3]. Thus, the possibility that the hair-cell synapse, or perhaps even other synapses, use a different molecule for calcium sensing is intriguing.

Second, all synaptotagmin molecules have so-called C2 domains, putative calcium-binding regions that are proposed to be responsible for its calcium-sensing functions[4,5]. Otoferlin contains six of these C2 domains, presumably for the binding of calcium, but it remains to be seen if or how other parts of the otoferlin molecule contribute to the unique properties of calcium-dependent signalling by the cochlear inner hair cell. Third, the new work raises the question of whether otoferlin or related molecules have a function at conventional synapses. Ferlin family members in non-neuronal cells have been identified as participants in membrane fusion events related to membrane repair[6].

Finally, Roux and colleagues' experiments[1] show how difficult it is to ascribe specific functions to molecules essential to synaptic-vesicle cycling. It is puzzling that the inner hair cell

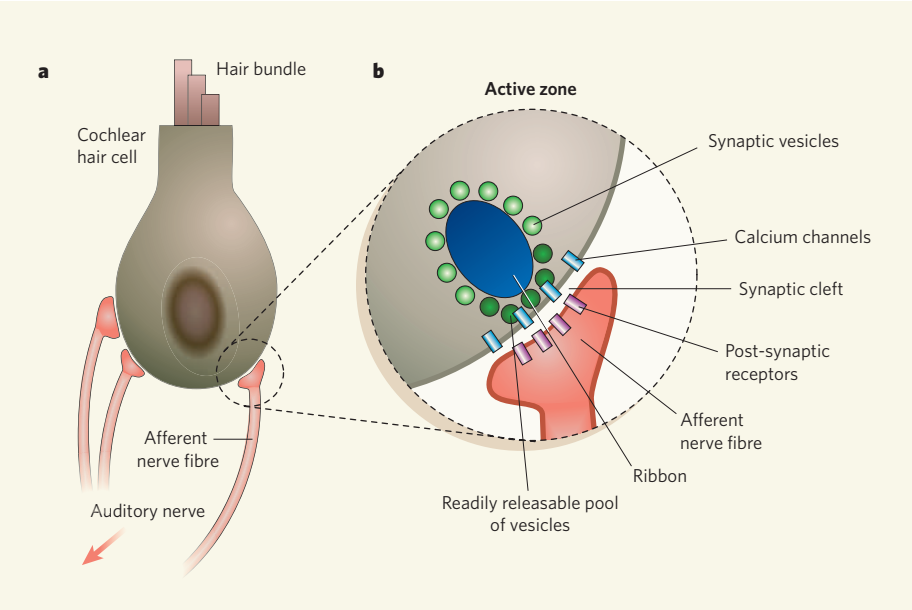

Figure 1 | Sound reception and perception. a, In the mammalian ear, the hair bundle on a cochlear hair cell senses variations in sound-induced pressure in the cochlea. The resulting, voltage-dependent signal is transduced and passed via the afferent nerve fibre and the auditory nerve to the brain, where it is perceived as sound. **b**, The voltage-dependent signal in the hair cell controls calcium channels, prompting calcium influx from outside the cell. Consequent changes in the cell's active zone result in neurotransmitter release into the synaptic cleft. In the active zone, aggregates of neurotransmitter-containing vesicles are tethered to a structure called the synaptic ribbon, localized to concentrated sites of calcium influx. A subpopulation of these vesicles lies close to the hair-cell membrane and constitutes a readily released source of neurotransmitter. Roux *et al.*[1] show that otoferlin is essential for the function of the auditory ribbon synapse, probably through its calcium-binding ability.

ribbon synapse looks so normal in mice with such a profound functional deficit. Given the blockade of synaptic-vesicle fusion, you would expect docked vesicles to accumulate at the release sites, but Roux *et al.* found no difference between the number of vesicles in normal mice and that in mice in which otoferlin production had been knocked out. The abolition of the fastest component of neurotransmitter release suggests that otoferlin acts on the most molecularly 'mature' vesicles, those conferred with rapid kinetics. Thus, otoferlin must mediate one of the final steps in the signalling cascade. However, definitive evidence of otoferlin's role as a calcium sensor awaits mutagenesis experiments on its putative calcium-binding C2 domains. ■

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1. Roux, I. *et al. Cell* **127,** 277–289 (2006).
2. Chapman, E. R. *Nature Rev. Mol. Cell Biol.* **3,** 498–508 (2002).
3. Sudhof, T. C. *Annu. Rev. Neurosci.* **27,** 509–547 (2004).
4. Fernandez-Chacon, R. *et al. Nature* **410,** 41–49 (2001).
5. Rhee, J. S. *et al. Proc. Natl Acad. Sci. USA* **102,** 18664–18669 (2005).
6. Doherty, K. R. & McNally, E. M. *Trends Mol. Med.* **9,** 327–330 (2003).

PARTICLE PHYSICS

Neutrons radiating decay

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That neutrons can be transmuted to protons, electrons and antineutrinos through the process of beta decay is old hat. That photons sometimes also get in on the act was suspected, but until now never confirmed.

The neutron, the existence of which was inferred by James Chadwick[1] in 1932, was one of the first members of nature's extensive particle zoo to be discovered. Yet it is only now, more than 70 years later, that one particular decay mode of the 'free' neutron — that is, a neutron not bound into an atomic nucleus — has been observed. On page 1059 of this issue, Nico *et al.*[2] describe how they caught up with this remarkably elusive phenomenon.

The 'radiative decay' that the authors observe is a consequence of the theory of quantum electrodynamics (QED), which describes all processes mediated by the electromagnetic force. Together with quantum chromodynamics, which characterizes the 'strong nuclear' force, and the theory of the weak nuclear force, QED is one of the three pillars of particle physicists' 'standard model'. According to the theory, whenever one or more electrically charged particles are created in a particle decay, each one may from time to time emit a light particle, or photon, in a process known as inner bremsstrahlung. For a free neutron n — which is unstable, with a lifetime of some 900 s — this radiative process is written $n \rightarrow p + e^- + \overline{\nu}_e + \gamma$, where the decay products are (aside from a photon, γ) those present in the familiar weak-force-mediated beta decay of the neutron: a proton p, an electron e^- and a ghostly particle known as an electron antineutrino, $\overline{\nu}_e$.

Although the radiative decay mode had previously been observed in the decay of radioactive nuclei and many elementary particles, it had never been observed in the decay of the free neutron. Only an upper limit on its probability existed[3]. Two recent theoretical works[4,5] have predicted a branching ratio of about 3×10^{-3} for photon energies larger than 15 kiloelectronvolts (keV). This number indicates the fractional intensity of a particular decay mode compared with all possible decays; in other words, one would expect a photon to be produced in three out of every thousand neutron decays.

Nico *et al.*[2] observed the decay of neutrons — as is often the case in experiments with free neutrons — during their passage through the experimental apparatus. In order that the neutrons should spend sufficient time in the apparatus, 'cold' neutrons are typically used. These have energies in the range 0.1–1.5 millielectronvolts that correspond to velocities of 140–1,000 m s^{-1}. Neutrons this slow are only formed in the rare process in which thermal neutrons obtained from a nuclear reactor lose almost all of their energy in a single collision.

Nico and colleagues performed their experiments at the research reactor of the US National Institute of Standards and Technology in Gaithersburg, Maryland. Their set-up has three features that are crucial to observing the radiative decay of the neutron (Fig. 1). The first relates to the challenge of distinguishing the low rate of low-energy radiative-decay photons from the intense background rate of photons. This background rate is produced by the scattering and capturing of neutrons from the beam in the materials around the detectors. The authors sharply reduced the background count by covering the surfaces that could be hit by neutrons with materials containing lithium-6: only about 1 in 10,000 neutrons captured on lithium-6 give rise to a photon.

A second feature of the apparatus takes into account the fact that electrons produce photons when they are slowed down in a detector, in a process known as external bremsstrahlung. These photons are nearly indistinguishable from radiative-decay photons. Nico *et al.*[2] therefore counted electrons and photons with two separate detectors placed at opposite ends of a region permeated by a strong magnetic field. Because electrons, unlike photons, are charged particles, they spiral along these field lines, and so can be guided out of the neutron beam by a slight bend in the magnetic field towards one end of the apparatus. (The protons created in the neutron decay follow similar paths, and are counted in the same detector.) The distance between the electron and the photon detectors, as well as the shielding between them, significantly reduces not only the photon background in the electron detector, but also the number of external-bremsstrahlung photons created in the electron detector that can reach the photon detector.

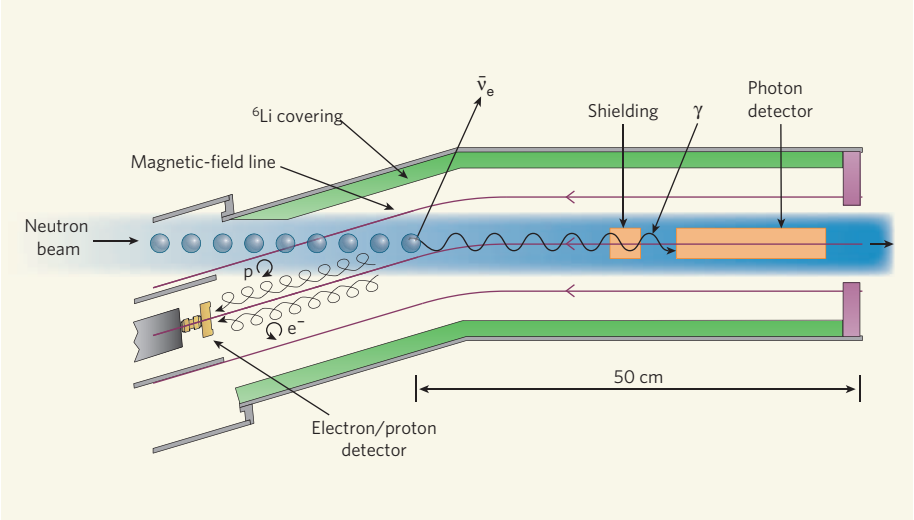

Figure 1 | Watching neutrons decay. Nico and colleagues' apparatus[2] included a number of features to ease the observation of the radiative decay of the neutron, including lithium-6-containing surfaces to minimize background photons, and separate detectors for protons and electrons, and for photons.