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Inner Ear: Ca^{2+}n You Feel the Noise?

Dispatch

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The death of hair cells in the inner ear as a result of exposure to loud noise can lead to irreversible deafness. New work shows that the mammalian cochlea can sense noxious sounds and use Ca^{2+} waves to rapidly propagate hair cell damage signals.

Hearing is one of our key senses and plays an important part in allowing us to understand our environment. In vertebrates, sound detection depends on the mechanosensory receptors in the inner ear called hair cells, which take their name from the hundred or so stereocilia making up the hair bundle that projects from the cellular apex [1]. Sound waves stimulate the hair bundle to increase and decrease the probability that mechanically gated transduction channels are in the 'open' state, with consequent changes in membrane potential [2,3]. There is, however, a problem with this exquisitely sensitive system: loud sounds can be harmful to the delicate stereocilia and may even produce hair cell death. Hearing loss as a result of hair cell damage is irreversible in humans, because the mammalian inner ear is normally unable to generate new hair cells after birth.

Other — non-mammalian — vertebrate species can replenish damaged ears with new hair cells throughout their entire lifetimes [4–6]. In some of these species, the rate of hair cell production is accelerated upon exposure to noxious noise levels, suggesting that a monitoring mechanism in the inner ear can sense damage and respond accordingly. Why cannot we do the same? Is the adult mammalian ear hopelessly unable to produce new hair cells, or might it be able to do so if only it 'knew' that it needed to? An elegant study by Gale *et al.* [7], published in this issue of *Current Biology*, begins to shed some light on this problem, by showing that the mammalian cochlea can indeed sense hair cell death, and use Ca^{2+} waves to propagate damage signals across the inner ear epithelium.

Tokyo, Nagasaki, New York and Buenos Aires are all wonderful cities, but a recent report by the World Health Organization indicates that they also are the four noisiest on the planet. Noise pollution in modern urban settings is an increasing cause of permanent hearing impairment. According to the US National Institute of Deafness and Other Communication Disorders, more than 30 million people in the USA are exposed to hazardous noise levels of 90 decibels (dB) or more on a regular basis [8]. Occupational noise levels can be even higher and potentially more devastating. In Germany, for example, as many as 12–15% of all employed people are exposed to daily noise levels of 85 dB or more [9]. So the possibility of preventing, or even reversing, hearing loss due to hair cell damage has important clinical and social implications. But some very basic questions need to be asked before we attempt to repair damaged mammalian ears [10,11]. For example, is hair cell damage being noticed at all in the inner ear? This, by no means a trivial question, is what Gale *et al.* [7] set out to answer with their study.

A response to cell injury within a tissue requires neighboring cells to sense the damage. Generally, this entails intercellular communication, which is required to organize cellular processes locally to produce the changes responsible for repair and, possibly, cellular regeneration. Ca^{2+}-mediated signal transduction is a common way of translating extracellular stimuli into functional cellular processes [12]. In several systems, it has been shown that mechanical stimulation results in a rapid and transient elevation in intracellular Ca^{2+} that spreads quickly as a wave to nearby cells.

Pancreatic islet cells and glia are among the cells that have been shown to undergo this process [13,14]. Astrocytes — a variety of glia — have two modes of intracellular communication that produce Ca^{2+} waves, related to the relative position of the cells. At areas where astrocytes contact each other, Ca^{2+} waves propagate across gap junctions between them. This process may involve intracellular signalling by inositol 1,4,5-trisphosphate (IP_3), as well as Ca^{2+} [15,16]. Separated astrocytes can also propagate intracellular Ca^{2+} waves, thought to be mediated by diffusion of extracellular ATP molecules [17,18]. Glial cells use Ca^{2+} signaling as the main mechanism for eliciting a physiological response to environmental stimuli. This response can result in growth, differentiation and release of neuroactive substances. Gale *et al.* [7] hypothesize that perhaps a similar change in intracellular calcium concentrations ($[Ca^{2+}]_i$) could constitute a signal for hair cell mechanical trauma.

Using radiometric Ca^{2+} imaging on rat cochlear organ culture preparations, Gale *et al.* [7] recorded the release and spread of Ca^{2+} waves from mechanically damaged hair cells to surrounding supporting cells at a uniform and constant speed of 14 μm per second. This observation is beautifully illustrated in the supplementary videos. The authors went on to show that extracellular ATP is a primary mediator for the generation of the $[Ca^{2+}]_i$ waves using tried and true pharmacological approaches. Applications of apyrase, a drug that hydrolyzes nucleotide triphosphates to monophosphates, restricted the spread of the Ca^{2+} wave, suggesting that Ca^{2+} waves propagate in an ATP-dependent manner. Mathematical modeling, in conjunction with Ca^{2+} wave analysis, indicated that the Ca^{2+} waves could not be accounted for by passive diffusion of ATP, with the ATP diffusion coefficient ranging from 15 to 240 μm^2 per second. This observation allowed the authors to hypothesize that an active mechanism must operate to maintain a constant rate of Ca^{2+} propagation.

Gale *et al.* [7] also found that topical applications of ATP or UTP elicited $[Ca^{2+}]_i$ oscillations almost

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identical to those observed after mechanical or laser-mediated hair cell damage, suggesting the involvement of purinergic receptors acting via IP_3 production. To test this possibility, they incubated cochlear explants in thapsigargin, which inhibits Ca^{2+} transport into the endoplasmic reticulum and thereby irreversibly depletes the intracellular Ca^{2+} stores. Although ATP-induced extracellular Ca^{2+} responses were still seen, the changes in $[Ca^{2+}]_i$ were reduced and $[Ca^{2+}]_i$ waves were absent.

To test whether purinoreceptors of the G-protein-coupled P2Y class are present in the rat cochlea, Gale *et al.* [7] used the reverse-transcriptase polymerase chain reaction (RT-PCR) and found a positive signal indicating expression of four different P2YR genes. Interestingly, noise-induced upregulation of a particular purinoreceptor, P2XR2, has already been documented in the rat cochlea [19]. Enhanced P2XR2 signaling may decrease the hair cells' response to sound, reducing their sensitivity to potentially damaging sustained high noise levels. The finding that P2YRs are highly expressed around the Hensen's cells region suggests that purinergic signaling may mediate the rapid propagation of an extracellular ATP-dependent hair cell damage signal. The novelty of this finding is that the initial source of ATP is a recently damaged hair cell.

These interesting results answer one important question but raise many more. For example, can these responses occur after noxious noise exposures that do not lead to hair cell rupture? One would think that it would be preferable to begin to sense noise-induced damage before hair cell death. If this is the case, what would be the source of the extracellular ATP needed for the generation and propagation of the $[Ca^{2+}]_i$ waves? Gale *et al.* [7] discuss this matter very briefly, pointing out that a vesicular store of ATP is present in the stria vascularis of the cochlea [20]. Previous studies have described an increase in the ATP levels in the endolymph during noise exposure, hypoxia and metabolic stress [20]. It has also been shown that a 15 minute exposure to a 110 dB sound level can increase endolymphatic ATP concentrations to around 20 nM, enough to trigger a mild or very limited, perhaps 'preventive', hair cell stress signal. In their study, Gale *et al.* [7] linked an increase in extracellular ATP concentrations to changes in cytosolic Ca^{2+}.

What does this mean in terms of repair? The authors' choice of a rat cochlea is very appropriate, because it is precisely in mammals that hair cell loss is irreparable. This model system, however, did not allow Gale *et al.* [7] to test the link between $[Ca^{2+}]_i$ waves and hair cell regeneration. One potential way forward would be to analyze repair responses to Ca^{2+} waves using a model system that does show spontaneous hair cell regeneration, such as the bird, frog or fish. How noise affects the inner ear is an important problem that needs to be identified, analyzed, and understood. The work of Gale *et al.* [7] is a big step in the right direction towards that aim, as it persuasively shows that when cochlear hair cells sustain mortal damage, a message is sent to local supporting cells in the form of Ca^{2+} waves.

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