A choreography of intracellular Ca^{2+} and extracellular ATP to refine auditory nociceptors before hearing

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Our hearing ability is exquisite in terms of sensitivity, dynamic range, and frequency discrimination. However, this acuity comes at the price of high vulnerability to a range of adverse influences—including sustained loud sounds. One may therefore expect that the ear is equipped with an alert system that can help prevent hearing damage, akin to the nociceptive sensory system that safeguards us from undergoing tissue injury. Here, Ceriani *et al* reveal a complex signaling interplay of sensory and non-sensory cells that helps setting up such a system in development.

The EMBO Journal (2019) 38: e101980

See also: F Ceriani *et al* (May 2019)

How might such a nociceptive system be implemented in the inner ear? The answer apparently relates to a peculiar dichotomy of sensory cell functions in the mammalian cochlea: One population of sensory receptor cells, the inner hair cells (IHCs), transduces sounds and sends this information to the brain. In contrast, the second receptor class, the outer hair cells (OHCs), is endowed with motor function, which serves to enhance the IHCs' response (in particular to low-level sounds), through a process known as cochlear amplification. This sensor dichotomy is also mirrored in neuronal connectivity. Each IHC connects to a bundle of type I afferent neurons that convey all relevant auditory information, such as sound intensity across a vast

dynamic range, frequency, and timing. OHCs, although fulfilling motor function, also make contact with afferent neurons named type II afferents. These distinct afferents only account for about 5% of all auditory neurons in the cochlea—yet their extensive dendrites span hundreds of microns to form synapses with dozens of OHCs (Weisz *et al*, 2012). The function of type II afferents has remained enigmatic. Only recently, complementary approaches to uncover their activity led to the idea that these afferents may be auditory nociceptors exclusively activated by noxiously loud sounds, possibly going along with tissue damage in the cochlea (Flores *et al*, 2015; Liu *et al*, 2015). Behavioral responses or sensations driven by this pathway remain unknown (Flores *et al*, 2015).

In most sensory systems, refinement of neuronal connectivity during development involves patterned, spontaneous activity in the afferent circuitry (Blankenship & Feller, 2010). In keeping with this principle, the developing auditory afferent pathway is spontaneously active, even before the cochlea is actually sensitive to sound (Tritsch *et al*, 2010). In rodents, spontaneous activity occurs during postnatal development before the onset of hearing. Seminal work has pinpointed a major origin of this pre-sensory activity to a non-sensory structure, Kölliker's organ (KO), a transitional epithelial ridge adjacent to the hair cells in the developing cochlea (Tritsch *et al*, 2007). In KO, spontaneous release of ATP induces local Ca^{2+} waves through purinergic

signaling, which ultimately excites or coordinates action potential firing in immature IHCs, in turn driving type I afferents through synaptic release of glutamate (Tritsch *et al*, 2010; Johnson *et al*, 2011). Although the importance of this spontaneous activity is not fully understood, it appears to be critical for proper wiring of central auditory circuits downstream of type I afferents (Wang & Bergles, 2015) as well as for development of the presynaptic function of IHCs in the auditory periphery (Johnson *et al*, 2017).

Given that type II afferents are only activated by noxious stimuli and hence expected to be silent throughout development, is their refined innervation pattern entirely predetermined? Based on three key findings, Ceriani and colleagues now propose that this may not be the case (Ceriani *et al*, 2019): First, using Ca^{2+} imaging on isolated cochlear preparations, they observe Ca^{2+} spike activity in OHCs during a narrow pre-hearing time window. As this spontaneous activity of postnatal OHCs had been essentially overlooked so far, this finding establishes a previously unknown drive for type II afferents during development. Secondly, they find that Ca^{2+} waves originating in KO enhance and temporally synchronize Ca^{2+} spiking in developing OHCs. The connection between KO and OHCs is provided by Deiters cells, supporting cells located below the OHCs. Notably, while pre-sensory activity is driven by Ca^{2+} waves in KO both for IHCs and for OHCs, the underlying cellular communication is fundamentally different:

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DOI 10.15252/emboj.2019101980 | Published online 11 April 2019

Figure 1. Model for signaling underlying refinement of type II afferent innervation of OHCs.

Model for signaling underlying refinement of type II afferent innervation of OHCs: Spontaneous Ca^{2+} waves emerge in Kölliker's organ and travel to Deiters cells underneath the OHCs, which in turn release ATP. ATP acts on P2X receptors of OHCs within the area excited by the Ca^{2+} wave to depolarize and electrically synchronize this group of OHCs. The resulting synchronous Ca^{2+} spikes in OHCs then instruct the maturation of OHC-to-type II neuron synapses, possibly by synaptic release of glutamate.

Ceriani *et al* show that enhanced OHC activity is driven by ATP release from Deiters cells acting on purinergic P2X receptors. In contrast, IHCs are activated by K^{+} ion efflux from their neighboring supporting cells (Wang *et al*, 2015). Thirdly, the authors find that type II innervation of OHCs is substantially impaired in a mouse model with reduced Ca^{2+} wave activity in KO. The role of OHCs in connecting Ca^{2+} waves to proper synaptic refinement is ascertained by another mouse model lacking $\text{Ca}_v1.3$ Ca^{2+} channels that drive afferent synapses in OHCs (Michna *et al*, 2003). In these mice, type II innervation was also impaired, phenocopying the mice with reduced Ca^{2+} waves.

Putting all these observations together, Ceriani *et al* propose a model (Fig 1) in which spontaneous Ca^{2+} waves in KO propagate into Deiters cells to release ATP. ATP in turn activates and synchronizes Ca^{2+} spikes in nearby OHCs through P2X receptor-dependent depolarization. Synchronous spiking of some ten OHCs hooked up to the same type II afferent is likely the signal driving type II activity required to establish mature innervation patterns. Ceriani *et al* provide insights into an unexpected, spatiotemporally complex intercellular choreography connecting Ca^{2+} signaling in non-sensory cells, purinergic signaling in OHCs, and type II afferents.

However, in astonishing contrast to these detailed developmental mechanisms, the

fundamental role of type II afferent activity and its neural and behavioral consequences still need to be established. In fact, it even remains to be figured out whether type II afferents are ever driven by OHCs in the hearing cochlea. With regard to developmental mechanisms, it is still unknown how the spontaneous activity in KO is generated and controlled.

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