News & Views

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Divide and conquer acoustic diversity

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Humans can recognize differences in sound intensity of up to 6 orders of magnitude. However, it is not clear how this is achieved and what enables our auditory systems to encode such a gradient. Özçete & Moser (2021) report in this issue that the key to this lies in the synaptic heterogeneity within individual sensory cells in the inner ear.

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See also: **Özçete ÖD & Moser T** (2021)

Six orders of magnitude in sound intensity separate the noise produced by the drop of a piece of cloth on a soft floor and the action of at least 3,000 W loudspeakers array. The question of how we can perceive such a broad range of sound intensities has brought attention to auditory scientists for decades. Pioneer studies have shown how first order neurons respond to simple acoustic stimuli (Kiang *et al*, 1965). One of the first observations was that these neurons, called spiral ganglion neurons (SGNs), were able to encode sound intensity; and not surprisingly, that they presented a basal firing rate that increased monotonically with the intensity of the stimulus. A saturation in firing rate was typically found when sound levels exceeded by < 10 times that of the threshold intensities. However, behavioral evaluations proved that individuals can recognize much broader differences in sound intensity. Insights came with the observation that not all SGNs are equal. These neurons were classified into three functional subtypes: "low spontaneous rate (SR) fibers", presenting scarce activity in the absence of sound; high-SR fibers, showing abundant activity in silence; and mid-SR neurons. Altogether, these three different subtypes of SGNs can cover the wide range of detectable sound intensities (Kiang *et al*, 1965; Liberman, 1978). However, the alternative way of referring to this heterogeneity of SGNs as high threshold to acoustic stimulation (low-SR) and low threshold (high-SR) would be more compatible with the physiological consequences of the problem. SGN diversity seems to hold for various mammalian species (for review, see, Heil & Peterson, 2015) and appears at all tonotopic regions of the cochlea.

Looking for the cellular bases of sound intensity encoding, Liberman (1982) found a peculiar innervation pattern of SGNs: high threshold (low-SR) fibers tend to contact IHCs from one side (called "modiolar" side) and low threshold (high-SR) fibers contact the same cell on the opposite face, or "pillar" side (see Fig 1). In other words, a single flask-shaped IHC of ~20 μm of length and 10 μm wide presents multiple synaptic contacts (between 10 and 20) with SGNs, all within an electrically and diffusionally compact volume (Liberman *et al*, 2011). IHCs are the primary receptors, converting graded changes in membrane potential into trains of action potentials in SGNs. Thus, how is it possible that a single IHC could drive the activity of these diverse groups of SGNs if all the functional synaptic contacts are governed by the same presynaptic membrane potential? Moreover, how can Ca^{2+} influx differ between release sites? If so, how is it that larger Ca^{2+} influx does not "contaminate" synaptic sites with smaller Ca^{2+} influx?

Some of these questions have received attention in the past, with important contributions made by the Moser group. In a previous paper, it was shown that larger active zones with stronger Ca^{2+} influx tend to reside on the modiolar side of IHCs, whereas pillar side synapses presented more hyperpolarized activation ranges for Ca^{2+} influx (Ohn *et al*, 2016). But some aspects of this problem remained an enigma to the field that has now been addressed by Özçete & Moser (2021). In the current study, the authors went one step further by simultaneously imaging, with a dual color approach, a specific Ca^{2+} indicator (Rhod-FF) and a fluorescent "glutamate sniffer", iGluSnFR, expressed in SGNs. This latter reporter has proved to be effective for visualizing glutamate release by neurons and astrocytes in different neuronal settings (Marvin *et al*, 2013), and now is applied for the first time in the cochlea. The great advantage of this approach is that individual synaptic contacts between one IHC and multiple SGN terminals can be imaged at once and allows for comparison of synapses on the modiolar vs pillar sides of the cell. The experimental setting requires good conditions for multiple parameters, such as high and homogeneous expression of iGluSnFR throughout a great number of SGNs, stable IHC recordings, and also stable signals from two different fluorescent probes that were imaged at high rates to correlate pre- and postsynaptic events (which are very fast by nature).

Özçete & Moser (2021) provide high-quality information on Ca^{2+} and glutamate signals from single synaptic sites as a function of IHC membrane potential, a way to mimic varying sound intensity stimuli. The authors found heterogeneity in the membrane potential at which Ca^{2+} and glutamate signals showed its half maximum activation ($V_{1/2}$). Chief among several findings is the observation that this heterogeneity was not simply random, but showed an interesting pillar-modiolar gradient, with lower signals at pillar sites (see Fig 1).

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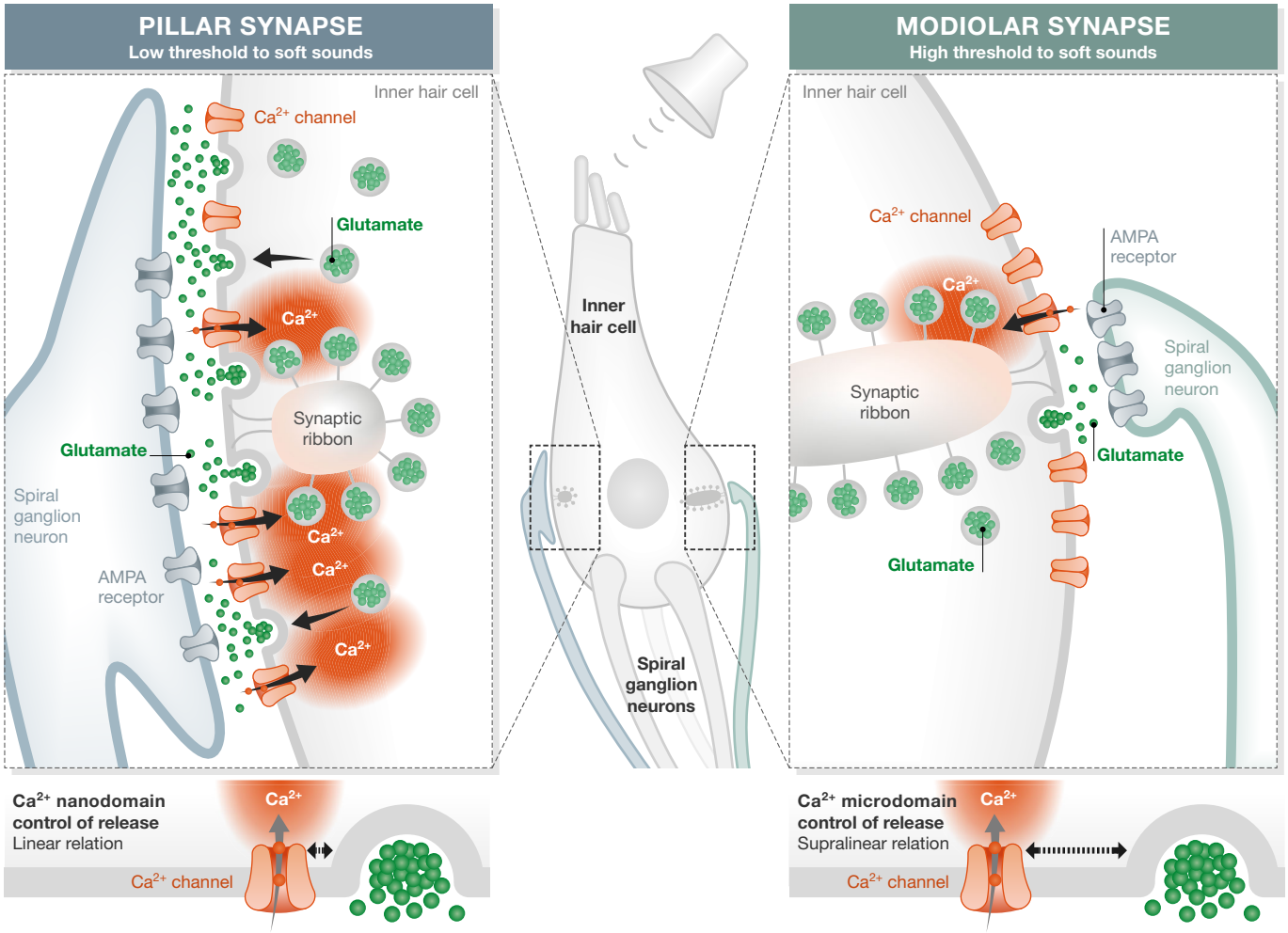

Figure 1. Individual IHCs are innervated by multiple SGNs forming single synaptic contacts.
In the study by Özçete and Moser, it is shown that synapses differ in multiple functional properties and can be classified into two main subtypes: low threshold synapses (typically on the Pillar side) and high threshold synapses (on the Modiolar side). Low threshold synapses are typically activated at lower IHC membrane potential (lower $V_{1/2}$), showing larger Ca^{2+} influx and more glutamate released. High threshold synapses present higher membrane potential activation (higher $V_{1/2}$). Low threshold synapses also presented a tight nanodomain coupling between Ca^{2+} channels and vesicles, whereas high threshold synapses tended to have a looser microdomain coupling.

What is the implication of this finding for hearing? Assuming that $V_{1/2}$ is a good proxy for SGNs threshold, this result correlates well with Liberman's findings in the early 80s showing that low threshold SGNs innervate IHCs on the pillar side (Liberman, 1982). The authors were also able to extract additional information from their rich dataset using principal component analysis. Most of the heterogeneity could be clustered in three groups with high resemblance to the classification made with respect to SGN spontaneous rates (Liberman, 1982), but also with a more recent description of genetic identities (Shrestha *et al*, 2018). One interesting observation is that the coupling between Ca^{2+} influx and glutamate release also varies among synapses. Chemical synapses have been classically described with one of two different modes of coupling: microdomain and nanodomain (Schneggenburger & Neher, 2005). The main difference between these two modes is the physical distance between Ca^{2+} sources and vesicles, which in the end also determines a cooperativity factor between Ca^{2+} influx and release. Whereas previous studies have shown that IHC present nanodomain coupling (Moser *et al*, 2020), the authors show now that within a given IHC different release modes can exist (see Fig 1).

However, interesting questions remain. Can this reported gradient in activation $V_{1/2}$ explain differences in SGN spontaneous rates as well? Are there additional factors determining spontaneous rates? Probably yes; intrinsic firing properties in SGNs may play an important role, together with the possible modulation by the axo-dendritic innervation of SGNs by centrifugal neurons of the olivo-cochlear system (Guinan, 2011). Also, how can individual synapses in each IHC diverge in $V_{1/2}$ or the cooperative coupling between Ca^{2+} and exocytosis?

This work from Özçete and Moser provides important information on long standing enigmas in the field and poses new questions for futures studies.

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