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Structure-function analysis of inner hair cell ribbon synapses

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The ribbon-type synapses between presynaptic inner hair cells (IHCs) and postsynaptic spiral ganglion neurons mediate the encoding of auditory signals into a neuronal code, in vertebrates. Acoustic information is transmitted with high temporal precision over long periods of time, requiring high rates of transient and sustained release.

Disruption of the synapse structure can result in deficits in neurotransmitter release that affect hearing function. Presently, we lack an integrated understanding of the molecular machinery regulating vesicle release at the presynaptic active zone (AZ) and transmission through the postsynaptic density (PSD). To approach this question, we study mutations disrupting synaptic proteins such as Otoferlin or RIM, taking advantage of recent advances in electron microscopy technology such as high-pressure freezing and electron tomography (Fig. 1).

We relate structures to functions to address the dynamics of synaptic vesicle pools and their proximity to active zone structures. With high-pressure freezing we compared inhibited versus stimulated, wild type versus mutant IHC ribbon synapses to determine parameters such as the number of membrane-proximal and ribbon-proximal synaptic vesicles and the synaptic vesicle diameter, depending on the activity state.

Moreover, we studied developmental changes upon synapse maturation (Fig. 2), showing that ribbon synapses undergo fundamental changes in structure and function when switching from presensory activity to graded sensory coding. In mature IHCs, one single large AZ apposes one large PSD. In contrast, before hearing onset, each IHC to spiral ganglion neuron synapse showed several pairs of smaller AZ/PSD complexes. Using a combination of electron, confocal, and stimulated emission depletion (STED) microscopy during development of hearing in the mouse, we demonstrate maturation of ribbon shape and anchorage. This was accompanied by a reduction of the number of extrasynaptic Ca^{2+}-channels and an increase in Ca^{2+}-channel colocalization with bassoon protein at AZs with tightly anchored ribbons.

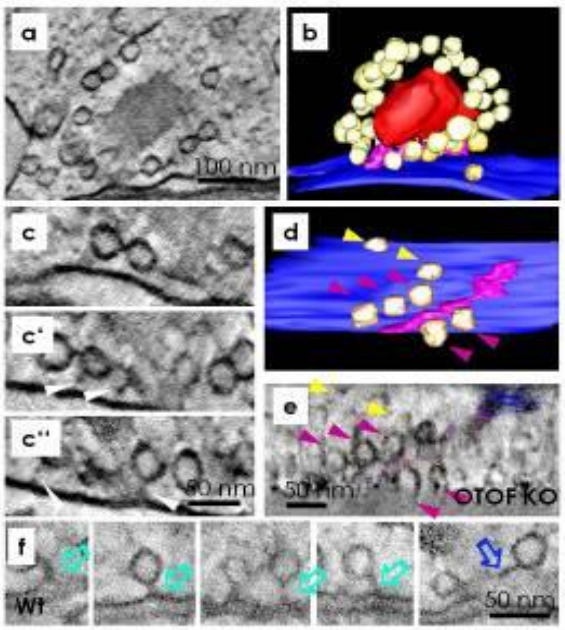

Figure 1: Ultrastructural details of ribbon synapses
(**a**) Electron tomogram (HPF, 250 nm section) of a typical OTOF KO ribbon synapse with the corresponding rendered model (**b**). (**c-c''**) Pool of membrane associated synaptic vesicles, connected via tethers to the membrane (white arrowheads), which are also observed at wild type synapses (**f**, arrows). (**d**) Model of membrane tethered SVs (arrowheads). Five SVs (red arrowheads) are arranged in rows next to the presynaptic density. (**e**) Tomogram according to (**d**), view generated using the slicer tool of the IMOD software package. Blue: active zone membrane; red: ribbon; magenta: presynaptic density; yellow: ribbon associated SVs; orange: membrane associated SVs.

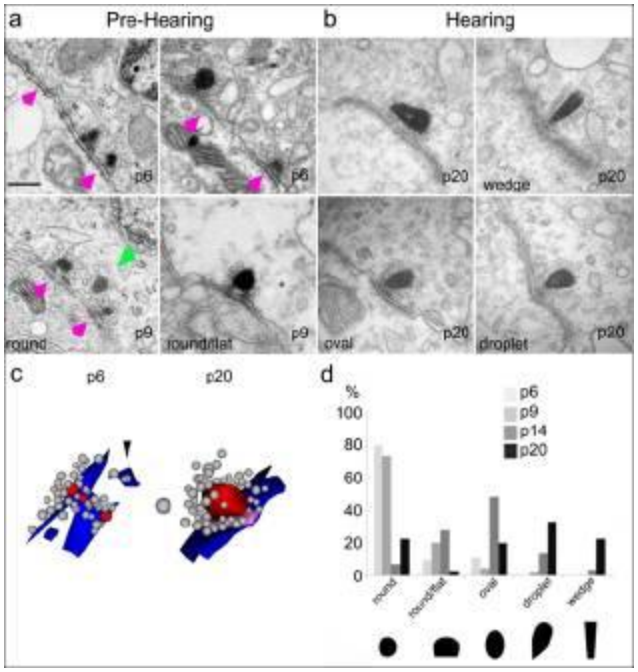

Figure 2: Maturation of inner hair cell ribbon synapses
(**a-b**) Representative electron micrographs of IHC ribbon synapses in pre-hearing (**a**) and hearing (**b**) mice. (**a**) Before the onset of hearing (p6 and p9) multiple appositions of small discontinuous pre- and postsynaptic membrane densities were found (magenta arrowheads), accompanied by one or more round-shaped ribbon(s). In some cases ribbons were "floating" (green arrowhead). (**b**) After the onset of hearing (p20) typically one ribbon occupied the presynaptic cytoplasm at the AZ and the mature pre- and postsynaptic densities were relatively extended and continuous. (**c**) 3D reconstructions from serial sections before (left, p6) and after (right, p20) the onset of hearing. Presynaptic rootlet: density connecting the ribbon the plasma membrane. (**d**) Development of the ribbon-shape. Scale bar in a: 150 nm.