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Hair cell regeneration in vestibular epithelia
-A study in an *in vitro* model

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Akademisk avhandling

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fredagen den 9 september, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

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Abstract
Background: Hair cells (HCs) are the sensory receptors in both the auditory and the vestibular organs of the inner ear. Supporting cells (SCs) are non-sensory cells embracing the HCs. Injuries of the HCs by ageing, acoustic trauma or ototoxic drugs (mainly aminoglycosides, e.g. gentamicin) and cisplatin, often cause permanent impairment of hearing and balance. Birds and amphibians can regenerate their auditory and vestibular HCs after injury through proliferation of SCs or direct transdifferentiation of a SC into a HC. For mammals this ability is limited and spontaneous HC regeneration occurs only in the vestibular sensory epithelia. The utricle is one of the five vestibular organs and contributes to our balance by registering linear acceleration and head tilts.
Aime: To investigate morphological and morphometric events during spontaneous HC regeneration following gentamicin exposure in neonatal rat utricular explants.
Methods: Long-term organ culture of macula utriculi, which is stable and reproducible for up to 28 days *in vitro* (DIV), was used in all papers in the thesis. HC damage was induced by gentamicin. On 2 DIV the explanted utricular maculae were divided into two groups, a control group and a gentamicin-exposed group. In the latter group macular explants were exposed to gentamicin for 48 hours during 2-3 DIV and then allowed to recover. Morphologic and morphometric evaluations were done from utricles harvested at various time points during 28 DIV. Imaging techniques used were light microscopy, including immunohistochemistry, and transmission electron microscopy.
Results: In the control group the epithelia were well preserved with a slight decline in HC density after 14 DIV. In the gentamicin exposed group there was an initial substantial decline in HC density and thereafter the proportion of HCs in relation to SCs increased significantly. Using BrdU as a proliferation marker and myosin 7a as a HC marker, we found no cells that were double marked. At the ultrastructural level, the apical occlusion of the explanted epithelia was intact in both the control and the gentamicin exposed group during the entire *in vitro* period. Cells that seemed to be in a transitional state, transforming from SCs into HCs were observed in the gentamicin-exposed group. These cells had cytoplasmic extensions basally i.e. foot processes, an assembly of mitochondria basally in the cell or in these foot processes, and often apical SC extensions covering the HC. HCs classified as transitional cells had an increased number of SC connections to their basal parts compared to mature HCs.
Conclusions: In these neonatal rat utricular explants: 1) The morphological structure of the sensory epithelia was well preserved. 2) The renewal of hair cells after gentamicin exposure occurred through direct transdifferentiation of supporting cells into hair cells. 3) There was also a proliferative response by the supporting cells, but this supporting cell proliferation did not contribute to the generation of new hair cells. 4) Cells in a transitional state, showing a characteristic morphology, were observed during the process of transdifferentiation from supporting cells into hair cells. 5) The tight junctional seal of the epithelia stayed morphologically intact also after gentamicin exposure. 6) Gap junctions were observed in between supporting cells but not found in between hair cells and supporting cells or between transitional cells and supporting cells.

Keywords
Vestibular hair cell; regeneration; transdifferentiation; proliferation: utricle; rat

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