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Using Zebrafish to Correlate Hair-Cell Activity with Ototoxin Resistance

Daria Lukasz[1,2], Katie Kindt[2]

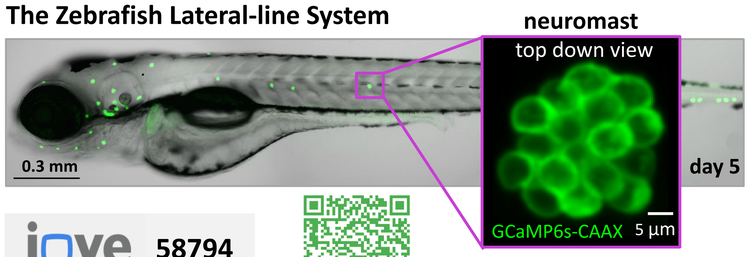
[1]Johns Hopkins University – National Institutes of Health Graduate Partnerships Program, Baltimore, MD
[2]National Institute on Deafness and Other Communication Disorders, Bethesda, MD

BACKGROUND

- Hair cells are the sensory receptors for hearing. In humans, they cannot regenerate once lost.
- Neomycin is a life saving antibiotic but is ototoxic and leads to hair-cell death and irreversible deafness[1].
- Persistent metabolic activity over time may sensitize hair cells to neomycin by contributing to a slow buildup of toxic metabolic byproducts and subsequent cellular damage[2].

METHODS

The Zebrafish Lateral-line System



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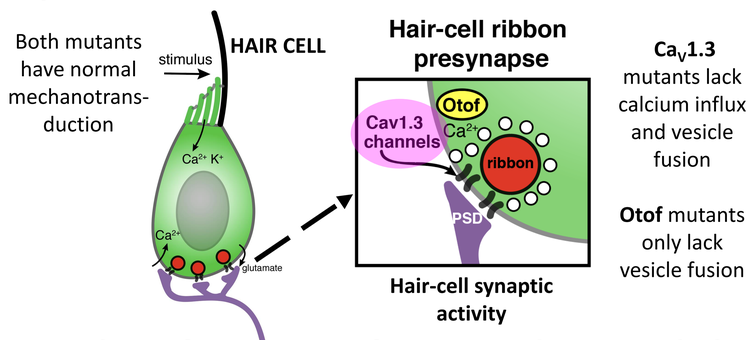
Zebrafish as a model system:

- Clusters of lateral line hair cells (neuromasts) are easy to access *in toto* and *in vivo*; simple bath application of drugs and dyes; tracking of individual cells
- Critical elements of hair-cell activity are conserved including: 1) $Ca_V1.3$, the channel required for presynaptic calcium influx and 2) Otoferlin, a sensor required to couple calcium influx to vesicle fusion

$Ca_V1.3^{-/-}$ and *$otof^{-/-}$* *Mutant* Hair Cells Lack Exocytosis

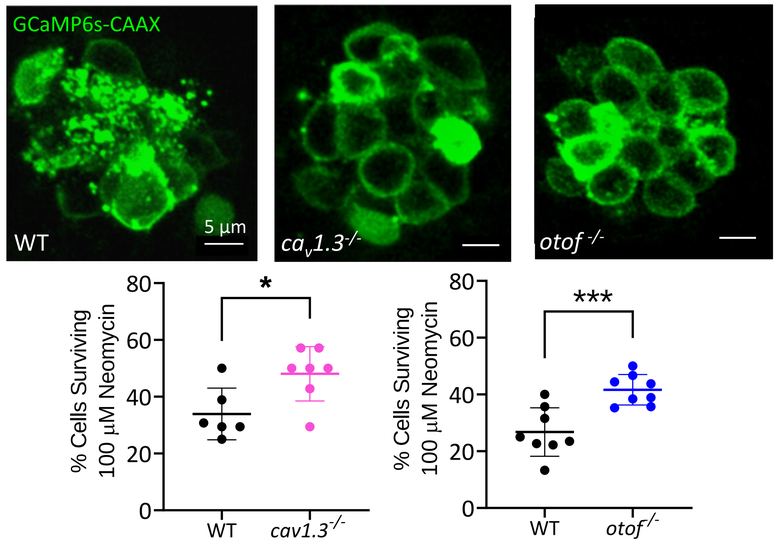


Does loss of presynapse function reduce metabolic demand and protect hair cells from neomycin?

HYPOTHESIS: Hair-cell presynaptic activity imparts susceptibility to neomycin-induced cell death.

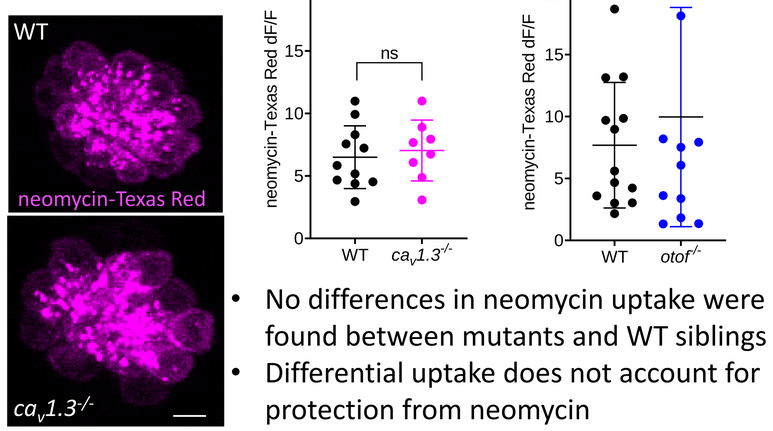
RESULTS

I. Loss of Presynaptic Activity Imparts Hair-Cell Neomycin Resistance



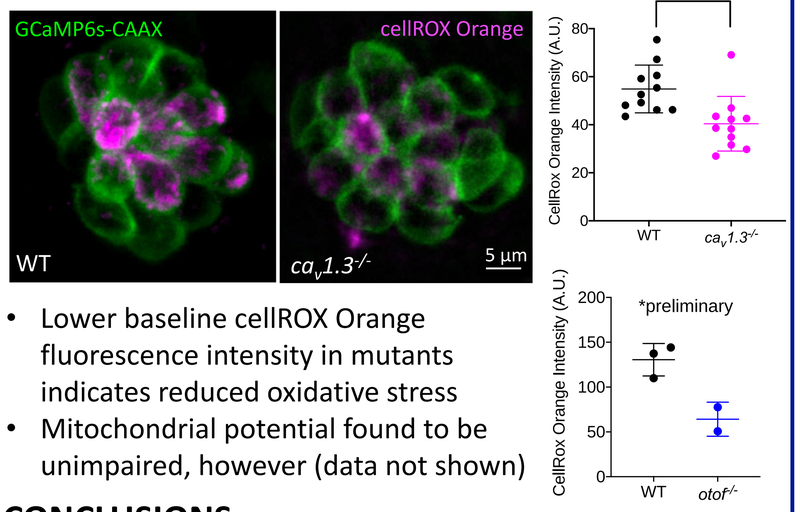
- Significant protection from neomycin is seen in *$ca_V1.3^{-/-}$* and *$otof^{-/-}$* mutants, suggesting that the metabolic demands of presynaptic activity may weaken hair cells over time
- Mutant neuromasts (above) look basically untouched after neomycin treatment (compare to untreated NM (left))
- Our next step was to confirm that differential neomycin uptake does not account for this protection (below)

II. *$Ca_V1.3^{-/-}$* and *$otof^{-/-}$* Mutants Have Normal Neomycin Uptake



- No differences in neomycin uptake were found between mutants and WT siblings
- Differential uptake does not account for protection from neomycin

III. Oxidative Stress is Reduced in *$ca_V1.3^{-/-}$* and *$otof^{-/-}$* Mutant Hair Cells



- Lower baseline cellROX Orange fluorescence intensity in mutants indicates reduced oxidative stress
- Mitochondrial potential found to be unimpaired, however (data not shown)

CONCLUSIONS

- Presynaptic activity sensitizes cells to neomycin ototoxicity likely due to the metabolic burden placed on mitochondria.
- Exocytosis may be a critical component of presynaptic activity that renders hair cells susceptible to neomycin because both presynaptic activity mutants lack exocytosis.
- ROS production and related oxidative stress associated with chronic presynaptic activity may weaken hair cells and make them more susceptible to neomycin

FUTURE DIRECTIONS

- Recent studies suggest that endocytosis in neurons is the most metabolically burdensome component of presynaptic activity[3].
- We will use the endocytic inhibitor Dynole 34-2 to transiently or chronically block endocytosis to further determine if perturbing endocytosis alone can bolster neomycin resistance and reduce metabolic stress.

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