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Predicting Profiles of Cochlear Pathology from Non-Invasive Physiological Biomarkers

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INTRODUCTION

Temporal bone histology shows that damage to outer hair cells, inner hair cells, the stria vascularis, and the auditory nerve contribute to sensorineural hearing loss[1]. Physiological measures such as otoacoustic emissions, the middle ear muscle reflex, and the auditory brainstem response are used in research and clinically to detect these pathologies and to determine a more precise site of lesion. However, it remains difficult to establish a one-to-one correlation between physiological responses and histological profiles, especially when more than one pathology is present.

In this study, we examined the relationship between histological profiles of cochlear pathology and responses on a battery of physiological tests sensitive to both OHC and non-OHC dysfunctions. Our preliminary results focused primarily on inner and outer hair cell counts across chinchillas with a variety of exposure histories.

METHODS

Animal Models and Exposure Conditions

Experimentally-Induced Hearing Loss		Unexposed
Noise Induced Temporary Threshold Shift (TTS) - Cochlear Synaptopathy[2] N = 9 animals	Noise Induced Permanent Threshold Shift (PTS) - Complex SNHL[3] N = 14 animals	**Control** N = 38 animals
Carboplatin (CA) Induced Inner Hair Cell Dysfunction[4, 5] N = 8 animals	Gentamicin Induced Outer Hair Cell Dysfunction[4] N = 5 animals	**Mystery Chinchillas** Older/High-Risk N = 3 animals

*N = number of animals included in physiological plots
See hair cell survival plots for histology N

All chinchillas were 6 months to 2 years of age, except for the "Mystery chinchillas" which were older animals (Q365: 6y;8m, Q368: 6y;7m, Q436: 4y;2m) who had been used for other experiments in the lab.

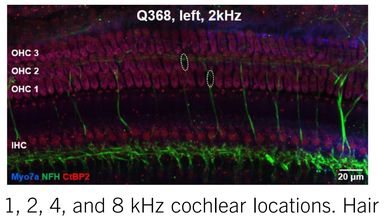
Biomarker Test Battery

ABR Thresholds .5, 1, 2, 4, 8 kHz	**Envelope Following Response[6] Rectangular Amplitude Modulated** 4kHz tone, 223 Hz modulation	**Distortion Product Otoacoustic Emissions**
Wideband Middle-Ear Muscle Reflex[7]	**Click ABR** High level stimulus	**Stimulus Frequency Otoacoustic Emissions**

Physiological assessment with the above battery was performed pre-exposure (results shown as Control Group physiology) and two weeks after exposure. Previous analyses showed that the average ABR threshold, average DPOAE amplitude, EFR magnitude, EFR phase locking value (PLV), MEMR strength (response at 105 dB FPL), and Wave 1 amplitudes were the strongest predictors on exposure group. Responses across exposure groups and individual "mystery chinchillas" are shown as a percent change in each measure relative to the average control measures.

Cochlear Histology

After all testing was completed, animals were euthanized and perfused. Cochleas were harvested, post-fixed, and decalcified. Cochleas were then bisected through the modiolus, immunolabeled, and dissected into half turns.

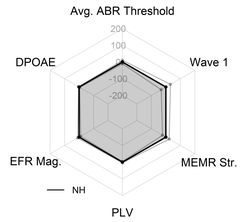


Specimens were labeled for:
- hair cells (Myo7a)
- afferent neurons (NFH)
- hair cell presynaptic ribbons (CtBP2)
- In a subset, stereocilia (ESPN)

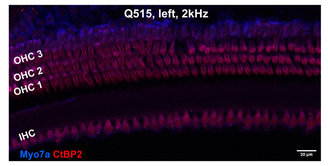
Confocal z-stack images were obtained at .5, 1, 2, 4, and 8 kHz cochlear locations. Hair cells counts were obtained at each of the 5 cochlear locations when dissection and image quality allowed. Hair cell survival is averaged across ears when both ears were available, otherwise, only data from one ear is shown.

RESULTS

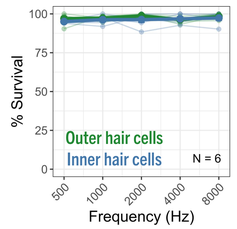
Control



Physiological test results were averaged across baseline measurements and visualized as a radar plot. Light lines represent the 95% confidence interval around the mean (same in all radar plots).



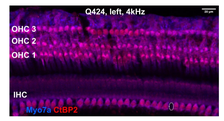
3 rows of OHCs and a single row of IHCs are present

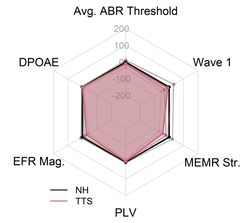


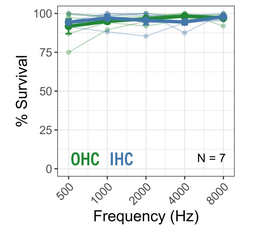
Inner hair cell and outer hair cell survival (present/absent) was quantified. Control animals show essentially no hair cell loss.

Cochlear Synaptopathy

TTS animals show reduced EFR magnitudes and reduced MEMR strength compared to baseline. OHCs and IHCs are broadly intact for all animals. Synapse counts are ongoing.

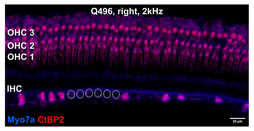




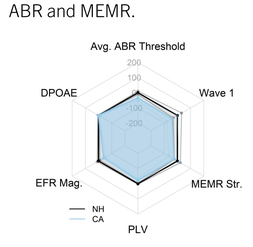


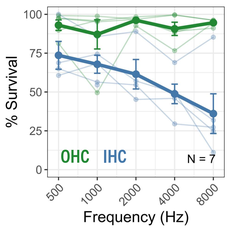
Inner Hair Cell Loss

Carboplatin results in significant IHC loss across the frequency range. Some frequency locations reveal missing OHCs. Both EFR magnitude and PLV are reduced in these animals, despite limited changes in ABR and MEMR.



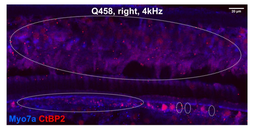
Some IHCs are missing and highlighted with the dotted white lines



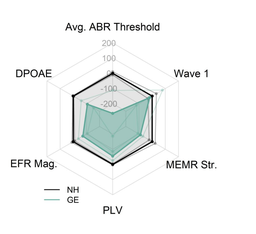


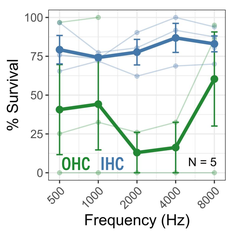
Outer Hair Cell Loss

Gentamicin exposure resulted in extreme OHC loss and some IHC loss. This damage was accompanied by significantly reduced ABR thresholds, DPOAEs, and EFR responses.



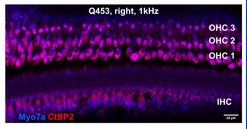
Large regions of missing OHCs and IHCs are highlighted here

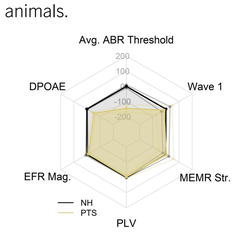


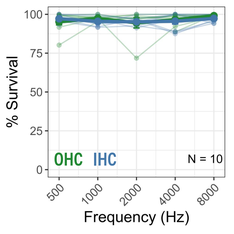


Complex Hearing Loss

NIHL reduced ABR thresholds and DPOAEs as expected. Despite 20-40 dB ABR threshold shifts, overt hair cell loss was limited. Although present, OHCs are less well-organized in some of these animals.



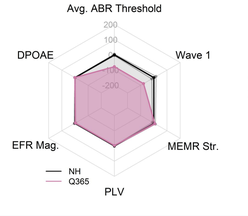


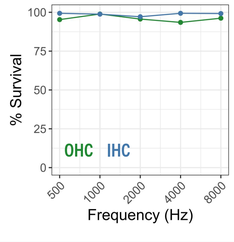


Mystery Chinchillas

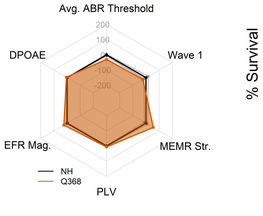
We also explored the relationship between histology and physiology at the individual level in a subset of animals without experimentally-induced hearing loss, but greater risk for sensorineural pathology given their age and noise exposure history.

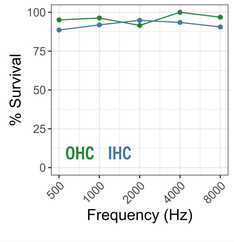
Chinchilla 1: Q365 Compared to control animals, ABR thresholds and Wave 1 amplitude were reduced without concurrent OHC and IHC loss (>90% survival).



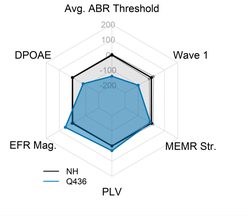


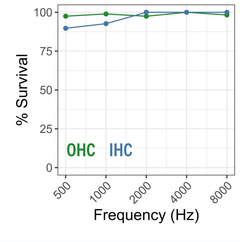
Chinchilla 2: Q368 IHC and OHC survival is similar to Q365 (>85% survival across frequencies), though biomarker responses are slightly stronger in this animal.





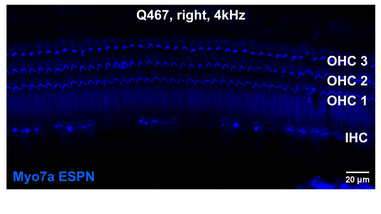
Chinchilla 3: Q436 Despite evidence of OHC dysfunction (reduced ABR thresholds, DPOAEs), there is no overt OHC loss at any of the analyzed frequencies.





FUTURE DIRECTIONS

Future datasets will be analyzed for other features such as synapses, auditory nerve fibers, and stereocilia.



Stereocilia from a carboplatin-exposed animal shows IHC stereocilia disarray in remaining cells.

CONCLUSIONS

- Hair cell survival alone is inadequate to account for the full range of pathology possible including more subtle effects such a stereocilia damage.
- This is especially important for predicting profiles at the individual level.
- These precision biomarkers show promise for non-invasively assessing inner ear health and appear to be capturing dysfunction not represented by hair cell presence.

REFERENCES

[1] Wu, O'Malley, de Gruttola, & Liberman, *J Neuro* 2020. [2] Kujawa & Liberman, *J Neuro* 2009. [3] Parida & Heinz, J Neuro 2022. [4] Axe, Purdue Univ., 2017. [5] Lobarinas, Salvi, Ding, *Hear Res* 2013. [4] Stone & Moore, *JASA* 2014. [6] Vasilkov, Garrett, Mauerman, & Verhulst Hear Res 2021. [7] Bharadwaj, Hustedt-Mai, Ginsberg, Dougherty, Muthaiah, Hagedorn, Simpson & Heinz, *Comm Bio* 2022.

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