Hidden Hearing Loss:
AMPA Receptor Mediated Cochlear Synaptopathy

Forrest Fearington[1], Tamasen Hayward[1], Allison B. Coffin[1]
[1]Neuroscience Program, Washington State University Vancouver

Background

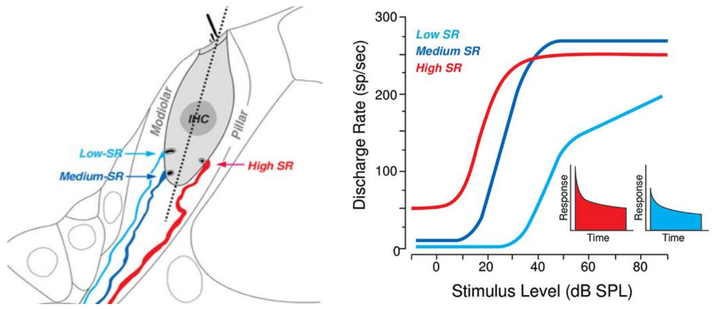
- Individuals with hidden hearing loss appear to have normal hearing thresholds per audiometric tests, yet they still have difficulty hearing and understanding speech in moderate background noise[1].
- Hidden hearing loss is suspected to be caused by synaptopathy, in which the synapses connecting sensory hair cells in our inner ear to afferent spiral ganglion neurons (SGN) are damaged.
- The exact mechanism of synaptopathy is unknown.
- One hypothesis postulates that intense noise exposure causes sensory hair cells to release high quantities of glutamate into the synapse. This leads to excess calcium influx and degeneration of the SGN.
- Low spontaneous-rate SGNs, which fire in response to loud noise and are integral in processing speech in noise, are affected most severely[2].
- Previous studies indicate that calcium may enter SGNs via AMPA receptors lacking the calcium-filtering GluA2 subunit[3].

Objectives

Explore the evidence surrounding the AMPA receptor-mediated theory of synaptopathy and investigate these receptors' role in mediating symptoms of hidden hearing loss.

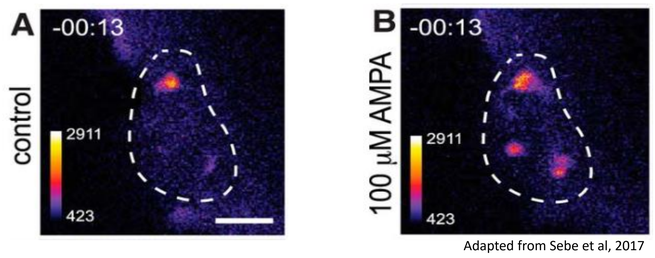
Cochlear Neurophysiology Basics

- Inner hair cells are innervated by low spontaneous rate (SR), medium SR, and high SR spiral ganglion neurons (SGNs).
- High and medium SR SGNs are quickly saturated at low decibel levels. Low SR continue graded response through high decibels and allow volume discernment[4].
- In hidden hearing loss, mostly low SR SGNs are damaged. Accounts for speech-in-noise complaints without change in auditory threshold[3,4].



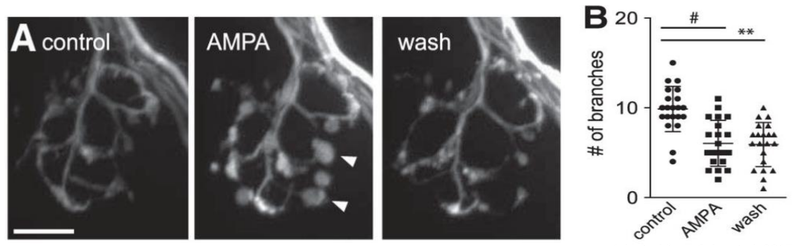
Bharadwaj et al, 2014

AMPA Receptors Leak Calcium Into the SGN



Adapted from Sebe et al, 2017

AMPA receptor agonist causes increase in SGN intracellular calcium, as measured via GCaMP transgenic zebrafish line.



Adapted from Sebe et al, 2017

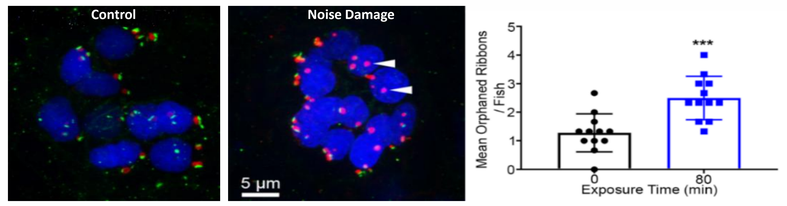
AMPA receptor agonist causes swelling and degradation of SGN dendrites, reflected in the reduction in dendritic branches.

AMPA receptors are composed of 4 subunits: GluA1, GluA2, GluA3, and GluA4. GluA2 acts as a filter that prevents calcium influx. If an AMPA receptor lacks a functional GluA2 subunit, it becomes calcium-permeable[6].

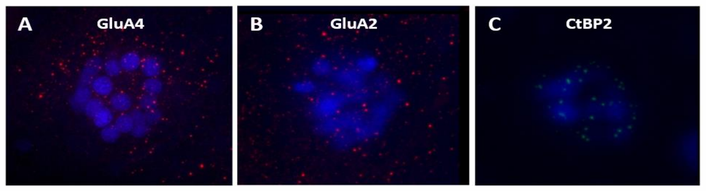
Next Steps

1) Use noise to damage zebrafish lateral line hair cells
2) Antibody label CtBP2 (ribbon synapse), GluA2, and GluA4 subunits.
3) Observe calcium influx into the ganglion cell in the presence of AMPA, DNQX (general AMPA antagonist), and IEM1460 (calcium-permeable AMPA antagonist).
4) Observe changes in CtBP2, GluA2, and GluA4 following synaptopathy.

Hypothesis: Noise will cause calcium influx through GluA2-lacking AMPA receptors. GluA2 distribution will be negatively correlated with calcium entry and synaptic damage in the ganglion cell.

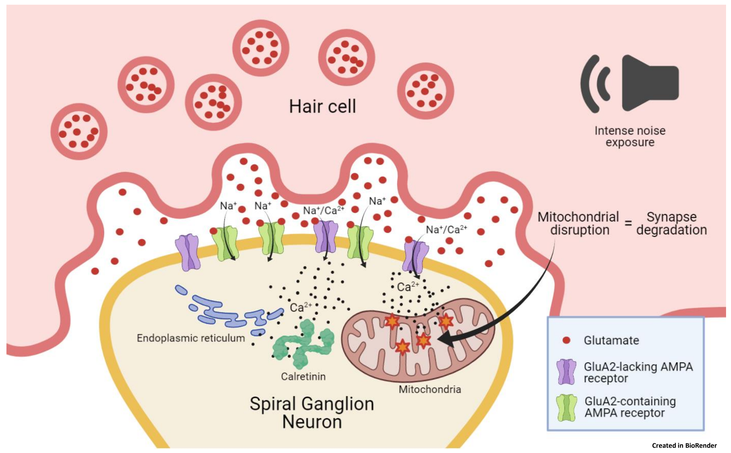


Noise damage causes postsynaptic SGN damage without loss of hair cell or presynaptic structures. Green = MAGUK (post synaptic density), red = Ribeye (presynaptic ribbon), blue = DAPI (hair cells). Uribe et al, 2018.



My successful antibody labeling in zebrafish with no noise damage. Hair cells labeled with DAPI (blue).

Summary of Hypothesized Mechanism



1) **Intense noise causes excess glutamate release**
2) **Glutamate binds to post-synaptic receptors**
3) **Calcium suspected to flow in through GluA2-lacking, calcium-permeable AMPA receptors**
4) **Normal calcium buffering mechanisms (endoplasmic reticulum, calretinin proteins, and mitochondria) are disrupted.**
5) **Mitochondria release apoptotic factors into cytosol, synapse degrades.**

Low SR SGNs have weaker calcium-buffering mechanisms (fewer mitochondria and fewer calretinin proteins)[5]. Therefore, they are the first to be damaged.

Conclusions

- Low SR SGN damage leads to intensity coding deficits characteristic of hidden hearing loss.
- AMPA agonists cause an increase in intracellular calcium, postsynaptic swelling, and loss of SGN dendritic branches.
- Synaptopathy can successfully be modeled using the zebrafish lateral line.

Remaining questions:

- **Is GluA2 subunit distribution correlated to calcium entry during noise exposure?**
- **How does AMPA receptor subunit composition change after noise damage?**
- **Can damage be prevented by blocking these receptors?**

Citations

1. Kujawa SG, & Liberman MC. (2009). Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *Journal of Neurosci*, *29*(45), 14077-85. doi: 10.1523/JNEUROSCI.2845-09.2009.
2. Liberman, L. D., Suzuki, J., & Liberman, M. C. (2015). Dynamics of cochlear synaptopathy after acoustic overexposure. *Journal of the Association for Research in Otolaryngology : JARO*, *16*(2), 205–219. https://doi.org/10.1007/s10162-015-0510-3
3. Sebe, J. Y., Cho, S., Sheets, L., Rutherford, M. A., von Gersdorff, H., & Raible, D. W. (2017). Ca2+-Permeable AMPARs Mediate Glutamatergic Transmission and Excitotoxic Damage at the Hair Cell Ribbon Synapse. *Journal of Neuroscience*, *37*(25), 6162–6175. https://ntserver1.wsulibs.wsu.edu:2137/10.1523/JNEUROSCI.3644-16.2017
4. Bharadwaj, H. M., Verhulst, S., Shaheen, L., Liberman, M. C., & Shinn-Cunningham, B. G. (2014). Cochlear neuropathy and the coding of supra-threshold sound. *Frontiers in Systems Neuroscience*, *8*, 26. https://doi.org/10.3389/fnsys.2014.00026
5. Sharma, K., Seo, Y.-W., & Yi, E. (2018). Differential Expression of Ca 2+ -buffering Protein Calretinin in Cochlear Afferent Fibers: A Possible Link to Vulnerability to Traumatic Noise. *Experimental Neurobiology*, *27*(5), 397–407. https://doi.org/10.5607/en.2018.27.5.397
6. Pachernegg, S., Münster, Y., Muth-Köhne, E., Fuhrmann, G., & Hollmann, M. (2015). GluA2 is rapidly edited at the Q/R site during neural differentiation in vitro. *Frontiers in Cellular Neuroscience*, *9*, 69. https://doi.org/10.3389/fncel.2015.00069
7. Uribe, P. M., Villapando, B. K., Lawton, K. J., Fang, Z., Gritsenko, D., Bhandiwad, A., Sisneros, J. A., Xu, J., & Coffin, A. B. (2018). Larval Zebrafish Lateral Line as a Model for Acoustic Trauma. *ENeuro*, *5*(4). https://ntserver1.wsulibs.wsu.edu:2137/10.1523/ENEURO.0206-18.2018

Funding: American Hearing Research Foundation, Hearing Health Foundation

16464