In Vitro and In Vivo Models to Test the Effects of Electrical Stimulation on the Inner Ear

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Introduction

- Cochlear implant electrode insertion can cause inner ear trauma resulting in loss of residual hearing.
- Recent advances in surgical technique and electrode design can help in preserving residual hearing.
- Loss of residual hearing may happen at during or after surgery:
  - Immediately after insertion of the electrode
  - Within the first month
  - After initial activation
  - Several months after activation
  - Kopelovich et al 2015 (5 patients with loss of RH post ES)
  - Eshraghi et al 2017 (decrease at 6000 Hz pre vs. post IS)
- Preservation of hair cells is even more crucial during the use of EAS or Hybrid cochlear implants
  - The effects of electrical stimulation on hair cells is not well known.
Mechanisms involved in the loss of residual hearing loss post-implantation

- Multiple mechanisms involved in loss of residual hearing:
  - Direct trauma by electrode - various macroscopic damages (graded 1 to 4)
  - Molecular damages
  - Activation of inflammatory cytokines, development of inner ear fibrosis, reaction to foreign body, hydrops, hydraulic trauma and vascular damage of lateral wall

CI electrode trauma grading scale

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<th>Severity of trauma:</th>
<th>Location of trauma:</th>
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<td>Grade 0 No observable macroscopic trauma*</td>
<td>A, lower basal turn; B, upper basal turn; C, lower middle turn; D, upper middle turn; E, apex.</td>
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<td>Grade 1 Elevation of basilar membrane</td>
<td>Any combination can be also presented, i.e. 4AB = lower and upper basal turn Grade 4 damage</td>
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<tr>
<td>Grade 2 Rupture of basilar membrane</td>
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<td>Grade 3 Dislocation of electrode in scala vestibuli</td>
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<td>Grade 4 Fracture of osseous spiral lamina or modiolus, or tear in tissues of stria vasularis/spiral ligament complex</td>
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*Possible damage at the molecular level that can lead to programmed cell death
Objectives

- To design a stimulator to test the effects of electrical stimulation on hair cells both *In-Vitro* and *In-Vivo*
Methods

STIMULATOR CONSTRUCTION

- High speed programmable current generators
- Floating stimulation electrodes: switches disconnect the electrodes during non stimulation period reducing stimulus artifact duration.
- Interface with commercial Evoked Potentials acquisition systems

- We can change multiple parameters to test a wide range of scenarios that a CI may encounter (Amplitude, Pulsewidth, Duration)

Providing the correct waveform in initial bench testing. Note the sharp, crisp response wave.

Stimulator turned to maximum power. Even though it is saturating, still a fast response time. This is far more power than we will need during testing.
Testing the otoprotective effect of a drug in an *in vitro* model of cochlear implantation trauma

- **Methods:** Organ of Corti dissected out from neonatal (P3-P5) rat pups, placed in microchannel slides inside incubator for 72 h

- We were able to modify various parameters of stimulation:
  - Amplitude, Pulsewidth, Duration

**Advantage:** Can test effects of different parameters as well as effects of various otoprotective therapies.

- Immunostaining with FITC, CellROX and cleaved caspase 3 staining to determine hair cell counts, the levels of reactive oxygen species (ROS) (oxidative stress) and apoptosis, respectively.

**Microscopy**
Electrical stimulation induces decrease in number of viable HCs at higher stimulation levels. There was further significant decrease in number of viable hair cells following increase in stimulation time period from 6 h to 24 h (P<0.001).

**Oxidative stress and apoptosis**

- Cochlear explants subjected to electrical stimulation exhibited positive CellROX and cleaved caspase 3 immunostaining.
- Treatment with the combination of Dex and L-NAC significantly reduced the CellROX labeling as well as cleaved caspase 3 immunostaining.
In-vivo Testing
eABR, ABR, MLR and DPOAE Recordings of a guinea pig with an implanted CI (with and without stimulation)
In-Vivo Results

- Higher fibrosis in cochleae from animals subjected to electrical stimulation

- Spiral ganglion neurons (SGNs) were decreased in animals subjected to electrical stimulation compared to control and EIT alone groups.

- We observed higher ABR threshold shifts in animals subjected to electrical stimulation compared to group

- Treatment with Dex and L-NAC significantly abrogated this increase in ABR threshold shifts (P<0.001).
DISCUSSION/CONCLUSIONS

- Our stimulator simulate well in-vitro and in-vivo the electrical field
- The electrical stimulation may causes damage to HCs, mainly with higher stimulation levels and longer times of stimulation.
- We were able to evaluate that our combination drug provided significant otoprotection against loss of hair cells.
- Our new novel experimental models will help in increasing our understanding of the role of electrical stimulation in the loss of Residual Hearing
- A better knowledge about the parameters of electrical stimulation that can affect cochlear sensory structures will pave the way to promote the preservation of residual and to test effective otoprotective approaches
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Thank you for your attention!