Current approaches to understanding vestibular hair cell regeneration using mouse models

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Regeneration in the Vestibular Periphery

Major points

1) Vestibular hair cells detect head motions, and vestibular afferent nerves relay signals to the brain that help us maintain our sense of well being

2) Vestibular sensory cells (hair cells) are impacted by different types of injury and by aging

3) Mature mice are good models to study regeneration. After damage, they replace key cell types (hair cells) and key structures (neurites & synapses) but this does not restore vestibulo-motor behaviors or reflexes
   
   Note: Mature cochlea lacks any type of regenerative response

4) Our studies focus on understanding natural regeneration and on promoting functional recovery using mouse genetics, cellular imaging, and behavioral/physiological testing
A functioning vestibular system is critical for our well being.

- Movements of eye, neck, and body
- Imbalance, vertigo, oscillopsia
- Oculomotor nuclei, spinal cord, cerebellum
- Vestibular Cortex
- Visual Proprioceptive Cerebellum
- Vestibular organs
- Vestibular nerve (afferent)
- Hippocampus
- CNX Nucleus
- Parabrachial Nuclei
- Amygdala
- Hypothalamus
- Vagus nerve
- Spatial orientation and memory
- Disorientation
- Assign subjective value to self-motion
- Anxiety and depression
- Blood pressure, heart rate, digestive tract
- Orthostatic hypotension, nausea

Modified from C. Balaban
Vestibular sensory pathway

Ango & Dos Reis, 2019
Causes of vestibular deficits

**PERIPHERAL**
- Developmental cochleovestibular anomalies
- Infections
- Tumors
- Benign positional vertigo
- Sensorineural damage (to hair cells, neurons, or both)

**CENTRAL**
- Vascular events (e.g., stroke)
- Concussive head trauma
- Neurodegeneration

Ango & Dos Reis, 2019
## Causes of sensorineural vestibular deficits

<table>
<thead>
<tr>
<th>Gene mutations</th>
<th>Ototoxic drugs</th>
<th>Exposure to intense concussive sounds (?)</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Gene mutations" /></td>
<td><img src="image2.png" alt="Ototoxic drugs" /></td>
<td><img src="image3.png" alt="Exposure to intense concussive sounds" /></td>
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<tr>
<td><strong>Aminoglycoside antibiotics</strong>&lt;br&gt;Gentamicin, streptomycin</td>
<td><strong>Anti-tumor drugs</strong>&lt;br&gt;Cisplatin</td>
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<tr>
<th>Microbial infections</th>
<th>Aging</th>
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<td><img src="image4.png" alt="Microbial infections" /></td>
<td><img src="image5.png" alt="Aging" /></td>
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<tr>
<td>CMV, Epstein Barr virus&lt;br&gt;Meningitis</td>
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Image Credits: EHA Rare Cancer Society UK, National Geographic, Sonic.Shield, www.copperheightsblog.com, Cleveland Clinic, scienceabc.com
~ 35% of people over the age of 40 experience vestibular deficits (Agrawal et al 2009)

Human vestibular hair cells degenerate with age
  Temporal bone autopsies (e.g., papers by S. Merchant and S. Rauch)
  Utricle biopsies (e.g., Taylor, Forge, et al 2015)
Why pursue vestibular hair cell regeneration as a therapy?

**Current treatment options** for sensorineural vestibulopathy:

- Prevent hair cell injury - Otoprotection

- Rehabilitative therapy - Promote substitution and adaptation
  
  *Substitution* uses other sensory systems – visual and somatosensory – to cope
  
  *Adaptation and compensation* reprogram brain through experience to make use of surviving vestibular pathway components

- Shortcomings of these options

- Insert vestibular implants - Directly stimulate vestibular nerves and bypass injured vestibular organs (under development)

**A potentially better option?** Restore the sensory organ

- Organs evolved to sense a wide range of head motions in different directions, speeds, and amplitudes in order to control multiple important bodily functions

- Sensory organ “rebuilding”, if effective, would restore more function to people
Mammals have 5 sensory organs for the vestibular system on each side of the body.

- **3 ampullae**
  - horizontal
  - anterior
  - posterior
  - Sense head rotations
  - Sensory epithelium = crista

- **2 otolithic organs**
  - utricle
  - saccule
  - Sense head tilt and linear motions
  - Sensory epithelium = macula
How do vestibular organs sense head position and motion?

**Stimulus:**
- Head acceleration (angular or linear)

**Gross response in ear:**
- Movement of fluid of semicircular canals or of the otoconia

**Sensory epithelial response:**
- Hair bundle (stereocilia) displacement
- Transduction currents > hair cell depolarization
- Synaptic transmission to vestibular afferent
Vestibular sensory epithelial structure (utricle)

Type I and II hair cells are
- Distributed across both zones
- Present in similar numbers
Two types of vestibular hair cells: Type I and type II

Both types respond to head motions
Both types susceptible to damage

Important differences

Type I hair cells are better suited to sense fast head motions

**Morphology**  
Hair bundle - more numerous stereocilia  
*Larger transduction currents*

**ePhysiology**  
Hair cell membrane conductances: $g_{K_{LV}}$ (low voltage)  
*Larger K+ conductances at lower membrane voltages*

Synaptic transmission: Quantal (glutamate) and non-quantal (ionic)  
*Faster synaptic transmission*
Morphology of vestibular afferents varies across zones in sensory organs

Fernandez et al 1990 (chinchilla)

Bouton-only
Extrastriola only
~2%

Calyx-only
Striola only
~6%

Dimorphic
All zones
~92%

Vestibular afferent neurite
Morphology of vestibular afferents varies across zones in sensory organs.

The predominance of dimorphic afferents makes it difficult to discern the specific functions of type I vs type II hair cells!
Steps toward developing hair cell regeneration as a therapy

Define the natural capacity for regeneration in adult mammals

Determine the extent to which natural regeneration restores function
   It is likely that new hair cells must:
   - acquire mature properties (mechanotransduction currents, bundle orientations, etc)
   - establish ample numbers of mature synapses with afferent neurons
   - be present in good numbers and in all zones of the organs

   It is likely that both type I and II hair cells will need to be replaced

Identify ways to overcome the shortcomings of our natural regenerative ability to promote full functional recovery
Anatomical evidence suggests humans can regenerate some vestibular hair cells

Taylor et al. 2015, 2018
Anatomical evidence suggests humans can regenerate some vestibular hair cells

Natural regeneration in humans must be very limited, though; people do not recover from substantial hair cell loss

Taylor et al. 2015, 2018
Mice are valuable models to study vestibular hair cell regeneration

**Experimental design**

Adult (6-9 week old) mice - wildtype (WT) or DTR heterozygotes

Two injections of DT @ 50 ng/g

Analysis of utricles and horizontal cristae

Days 0 2 7 14 42 70 140

Golub et al. 2012
Many vestibular hair cells are naturally regenerated in adult mice.
Many vestibular hair cells are naturally regenerated in adult mice.

The other vestibular organs have a similar degree of damage and regeneration.

Golub et al. 2012
In rodents, new hair cells are formed by supporting cells via a non-mitotic mechanism.

Mitotic regeneration

Non-Mitotic regeneration (direct transdifferentiation)

Depletion of supporting cells seems like a minor issue in adult mice.

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Birds and amphibians

Mammals
Regenerated hair cells are type II-like: Lack afferent calyx ending

Normal mice (cross section)  DTR mouse, 90 days post-DT (top-down view)

Myosin – hair cell
Neurofilament – afferent nerves

Golub et al. 2012
Regenerated hair cells are type II-like: Express the markers Calb2 and Sox2

Normal mice

DTR mouse, 21 days post-DT
All regenerated hair cells are type II-like (utricle)
New hair cells acquire appropriate bundle structure, and they mechanotransduce (utricle)

- Stereocilia height and diameter are within normal range
- Bundle asymmetry and orientations are normal
- Otoconial membrane and otoconia are intact

Transduction currents are smaller in regenerated hair cells

González Garrido et al. 2021
Vestibular ganglion neurons survive after hair cell destruction, and neurites remain in/return to the sensory epithelia.
Regenerated hair cells make ribbon synapses with vestibular afferents

González-Garrido et al. 2021
This degree of hair cell replacement does not restore vestibulomotor function

No recovery of vestibulo-motor behaviors
  Circling
  Head bobbing
  Failure to climb
Why does natural regeneration fail to restore balance function?

Regenerated hair cells:
- May be present in insufficient numbers
- May be too immature
- May not form functional synapses with vestibular afferents
- Only type II hair cells are replaced – no new type I hair cells