Current approaches to understanding vestibular hair cell regeneration using mouse models

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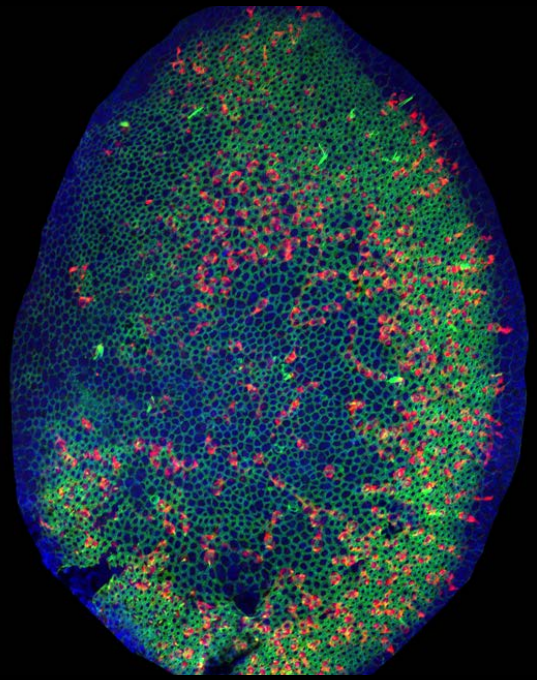
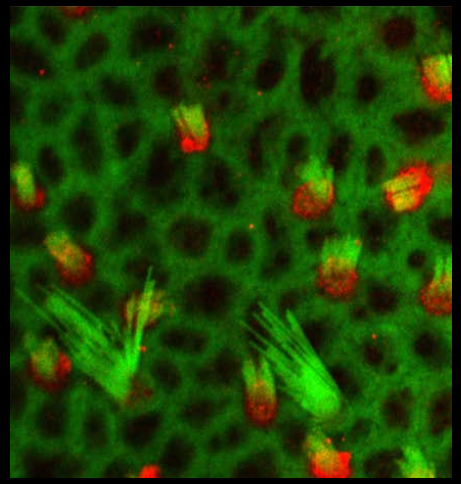
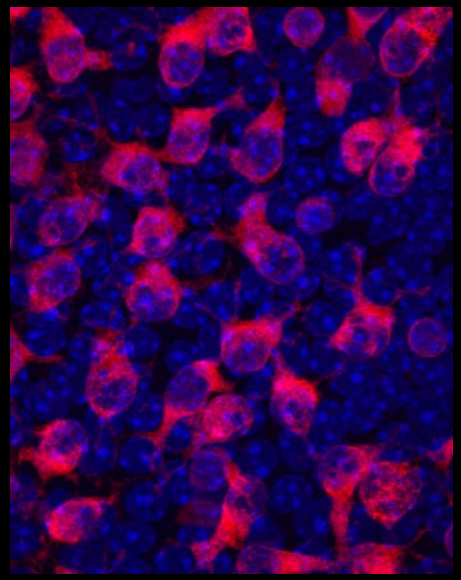
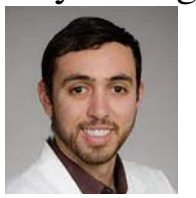






Acknowledgments

Kelli Hicks, Manny Jauregui, Hans Baertsch, Hendrik Dorssers, Tot Nguyen, Jim Phillips, Rémy Pujol

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Ruth Anne Eatock (U. Chicago)

Toni Gonzalez Garrido
Omar Lopez

Funding

NIH National Institute on Deafness and Other Communication Disorders (NIDCD)

Hearing Health Foundation
Prevention | Research | Cure

HRP Hearing Restoration Project

Virginia Merrill Bloedel Hearing Research Center

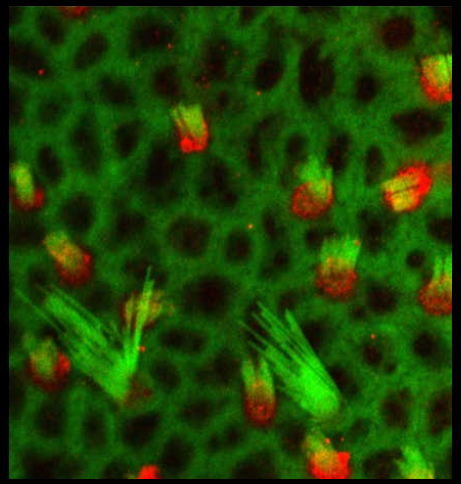
The Hamilton & Mildred Kellogg Charitable Trust

The Whitcraft Family

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



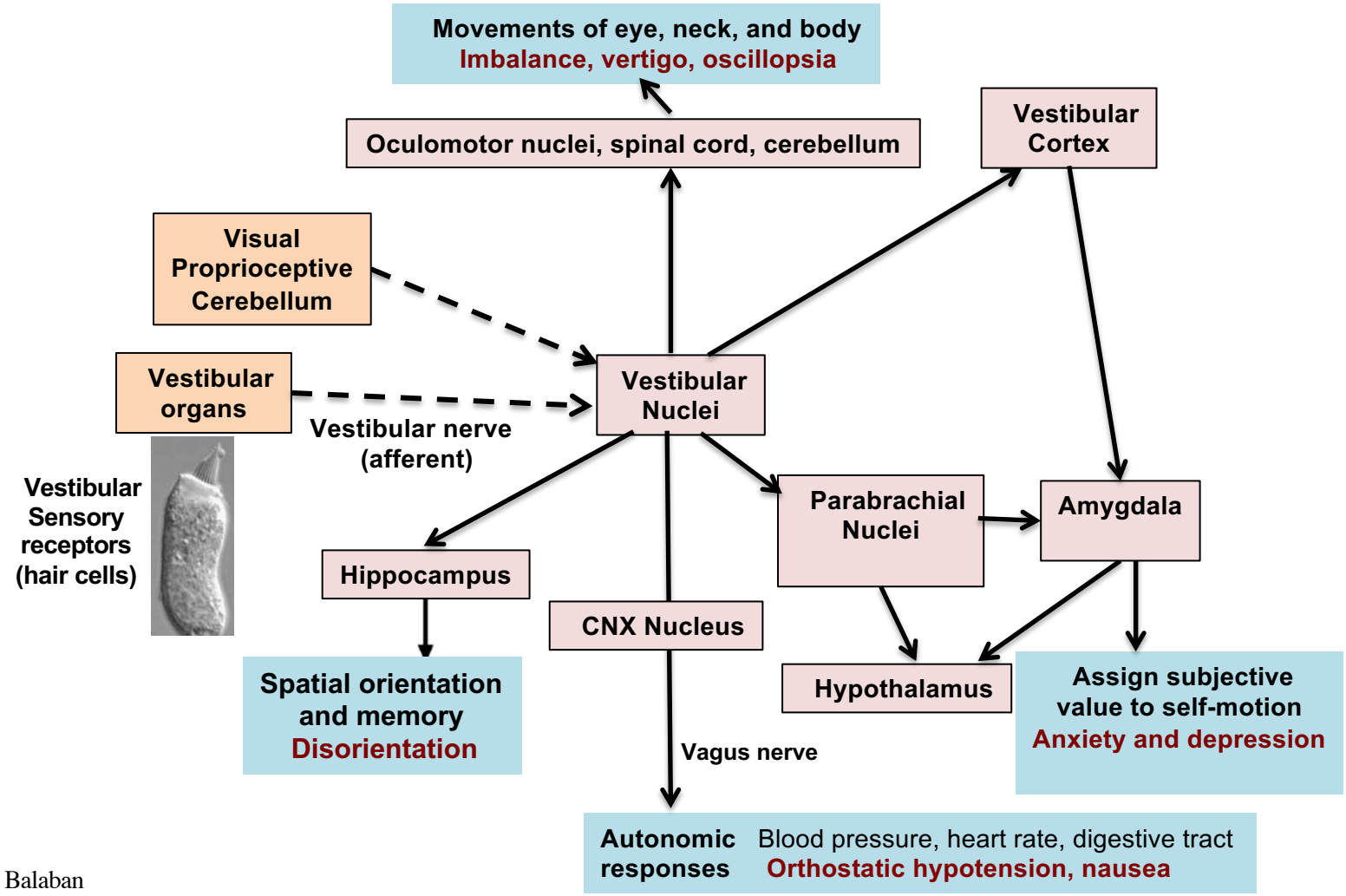
2) Vestibular sensory cells (hair cells) are impacted by different types of injury and by aging

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

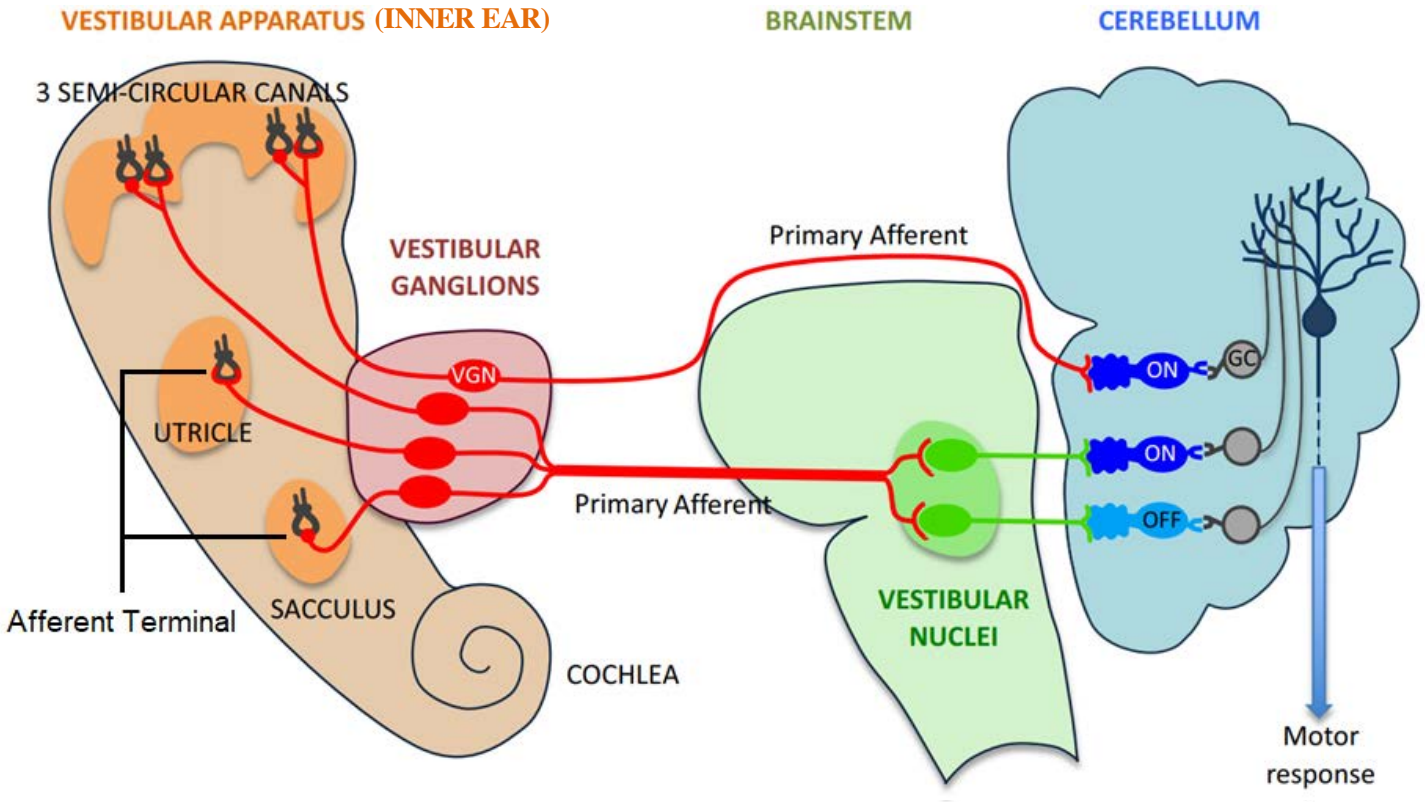
4) Our studies focus on understanding natural regeneration and on promoting functional recovery using mouse genetics, cellular imaging, and behavioral/physiological testing

A functioning vestibular system is critical for our well being



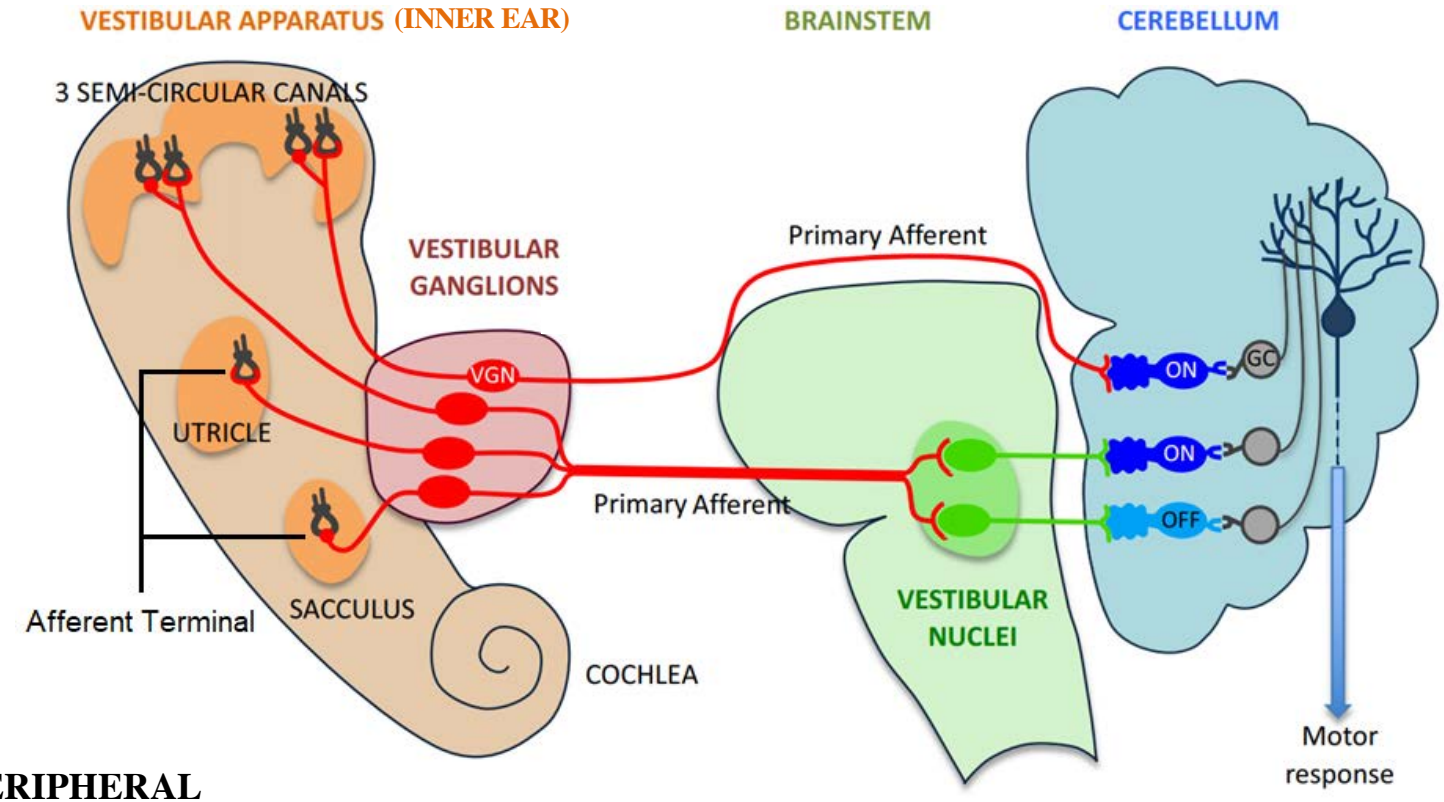
Modified from C. Balaban

Vestibular sensory pathway



Ango & Dos Reis, 2019

Causes of vestibular deficits



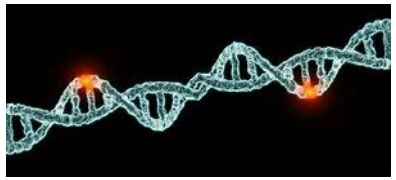
PERIPHERAL
Developmental cochleovestibular anomalies
Infections
Tumors
Benign positional vertigo
Sensorineural damage (to hair cells, neurons, or both)

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

Ango & Dos Reis, 2019

Causes of sensorineural vestibular deficts

Gene mutations

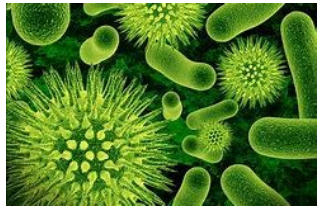


Ototoxic drugs

Aminoglycoside antibiotics
Gentamicin, streptomycin
Anti-tumor drugs Cisplatin

Exposure to intense concussive sounds (?)

Microbial infections



CMV, Epstein Barr virus
Meningitis

Aging

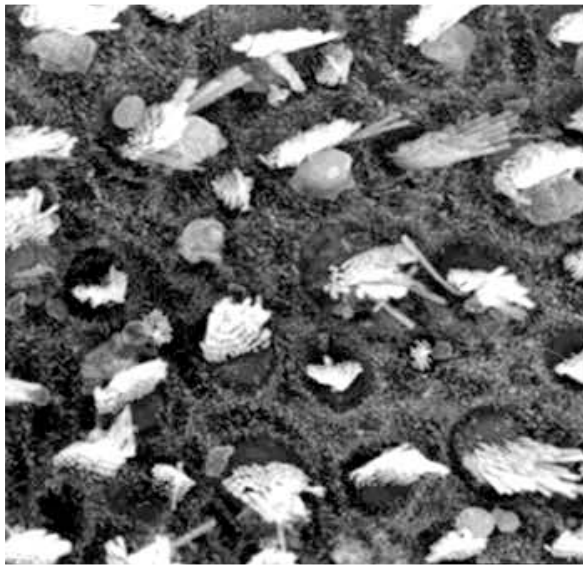
Image Credits: EHA Rare Cancer Society UK, National Geographic, Sonic.Shield, www.copperheightsblog.com Cleveland Clinic, scienceabc.com8

~ 35% of people over the age of 40 experience vestibular deficits (Agrawal et al 2009)

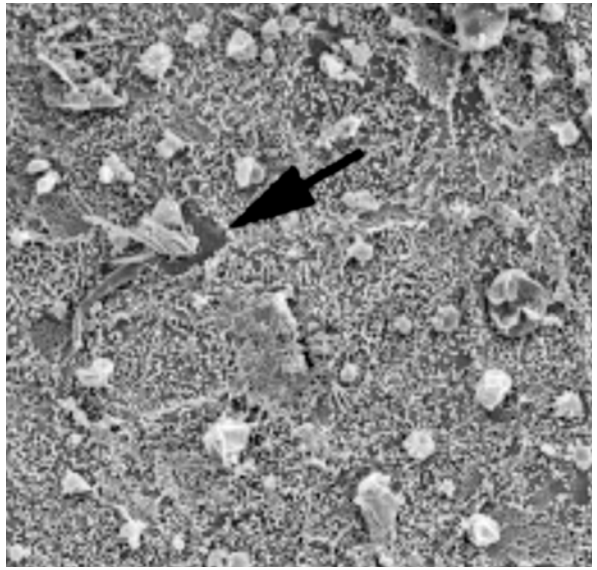
Human vestibular hair cells degenerate with age

- Temporal bone autopsies (e.g., papers by S. Merchant and S. Rauch)
- Utricle biopsies (e.g., Taylor, Forge, et al 2015)

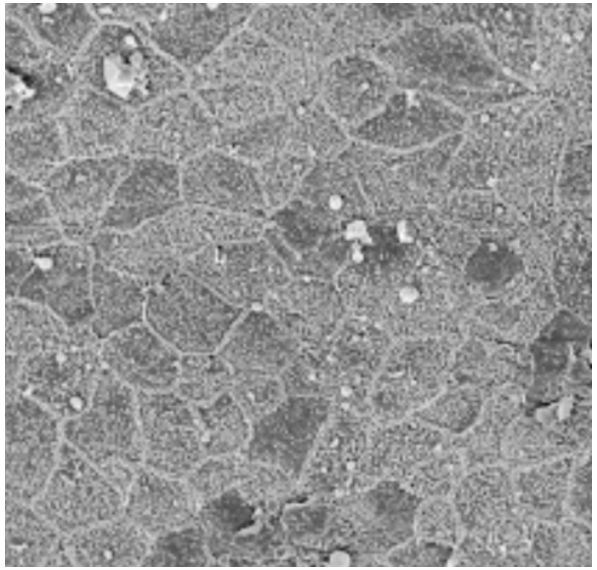
Guinea pig utricle



Human utricles



80 year-old woman



65 year-old man

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

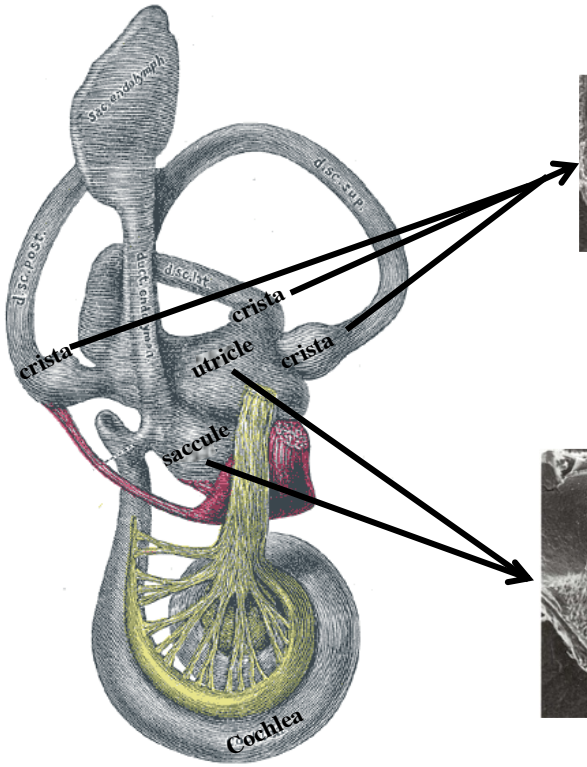
Insert vestibular implants - Directly stimulate vestibular nerves and bypass injured vestibular organs (under development)

A potentially better option? Restore the sensory organ

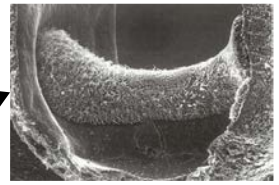
- Organs evolved to sense a wide range of head motions in different directions, speeds, and amplitudes in order to control multiple important bodily functions

- 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

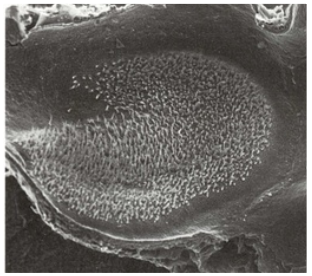


Membranous portion
of the inner ear



3 ampullae
- horizontal
- anterior
- posterior

Sense head rotations
Sensory epithelium = crista



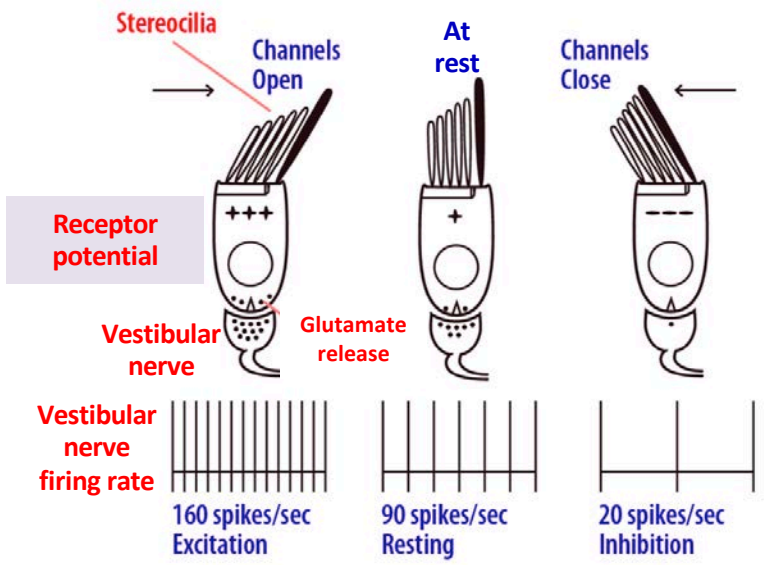
2 otolitic organs
- utricle
- saccule

Sense head tilt and linear motions
Sensory epithelium = macula

Gray's Anatomy, *Hunter-Duvar*

How do vestibular organs sense head position and motion?

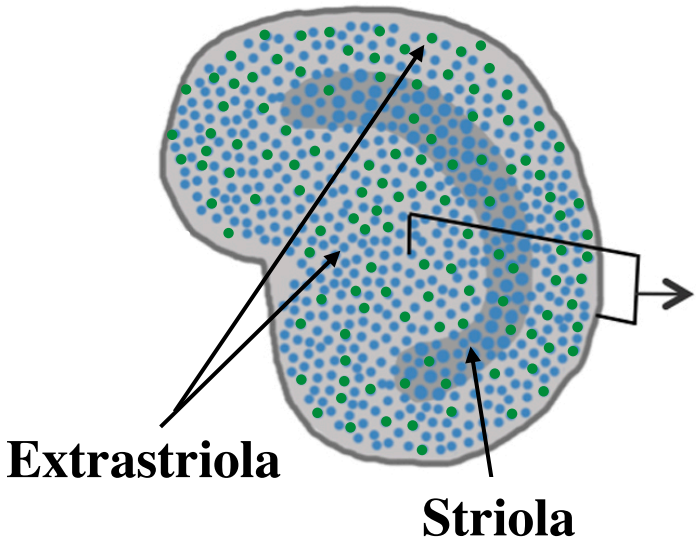
Stimulus:		Gross response in ear:		Sensory epithelial response:
Head acceleration (angular or linear)	>>>>>	Movement of fluid of semicircular canals or of the otoconia	>>>>>	Hair bundle (stereocilia) displacement Transduction currents > hair cell depolarization Synaptic transmission to vestibular afferent



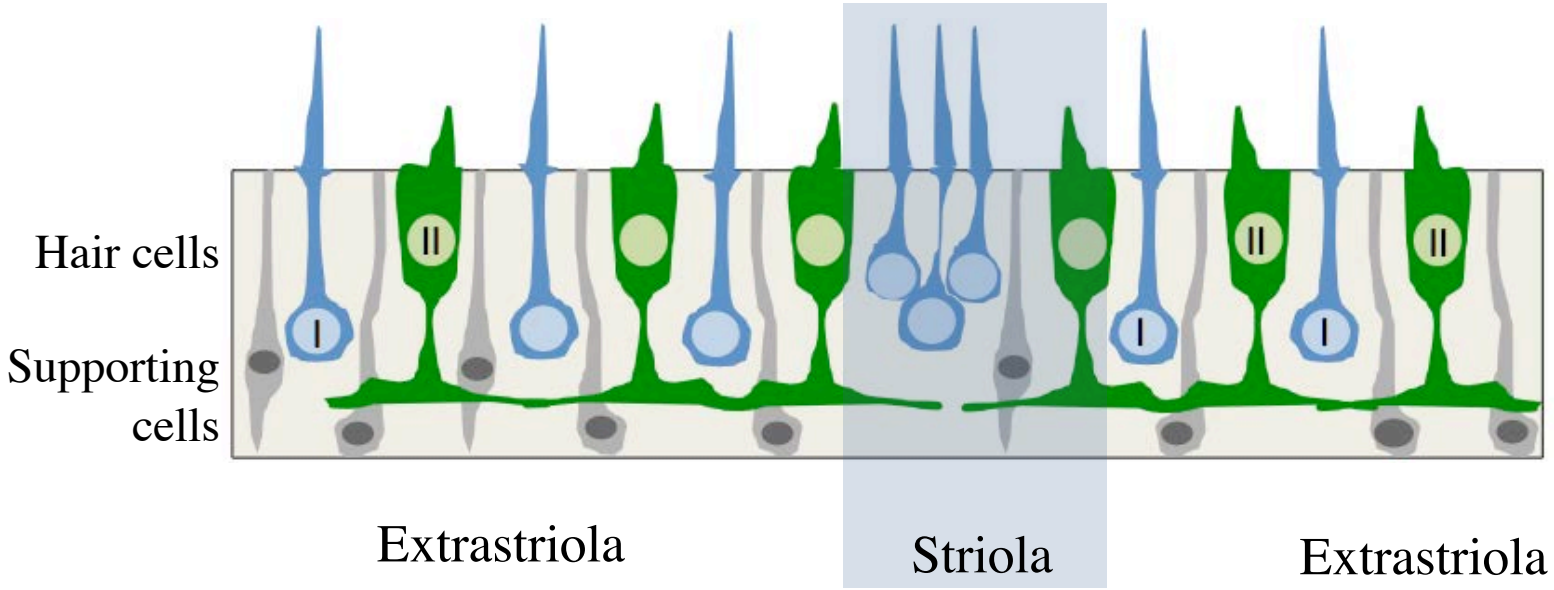
Dickman & Angelaki

Vestibular sensory epithelial structure (utricle)

Top-down view



Cross section



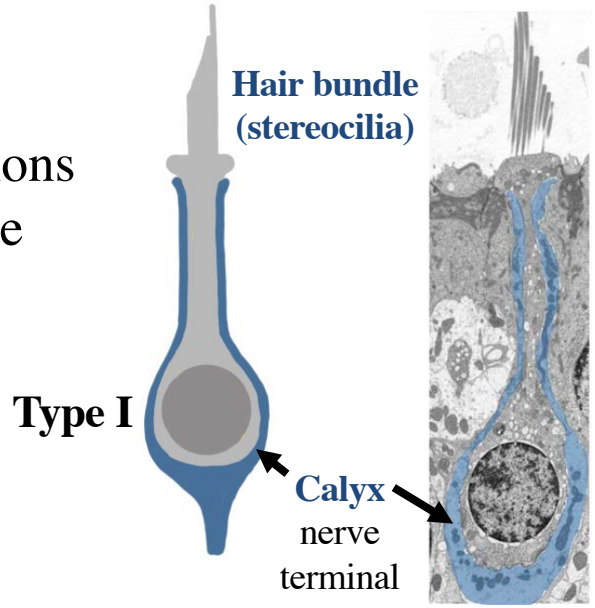
Type I and II hair cells are

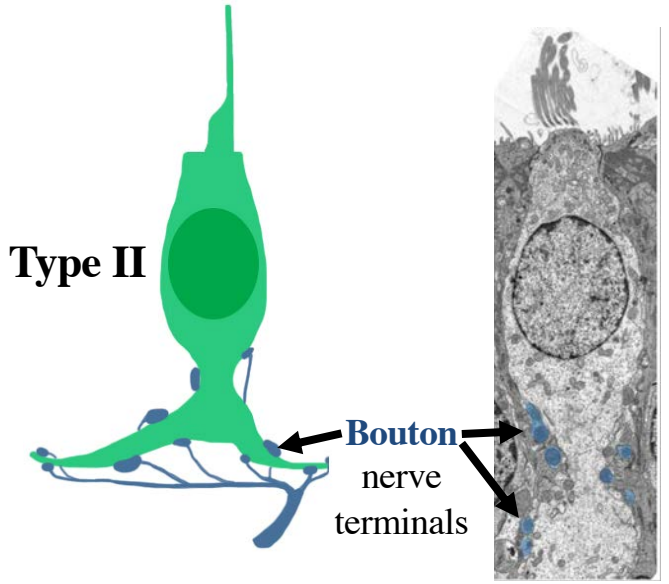
- Distributed across both zones
- Present in similar numbers

Two types of vestibular hair cells: Type I and type II

Both types respond to head motions
Both types susceptible to damage

Important differences





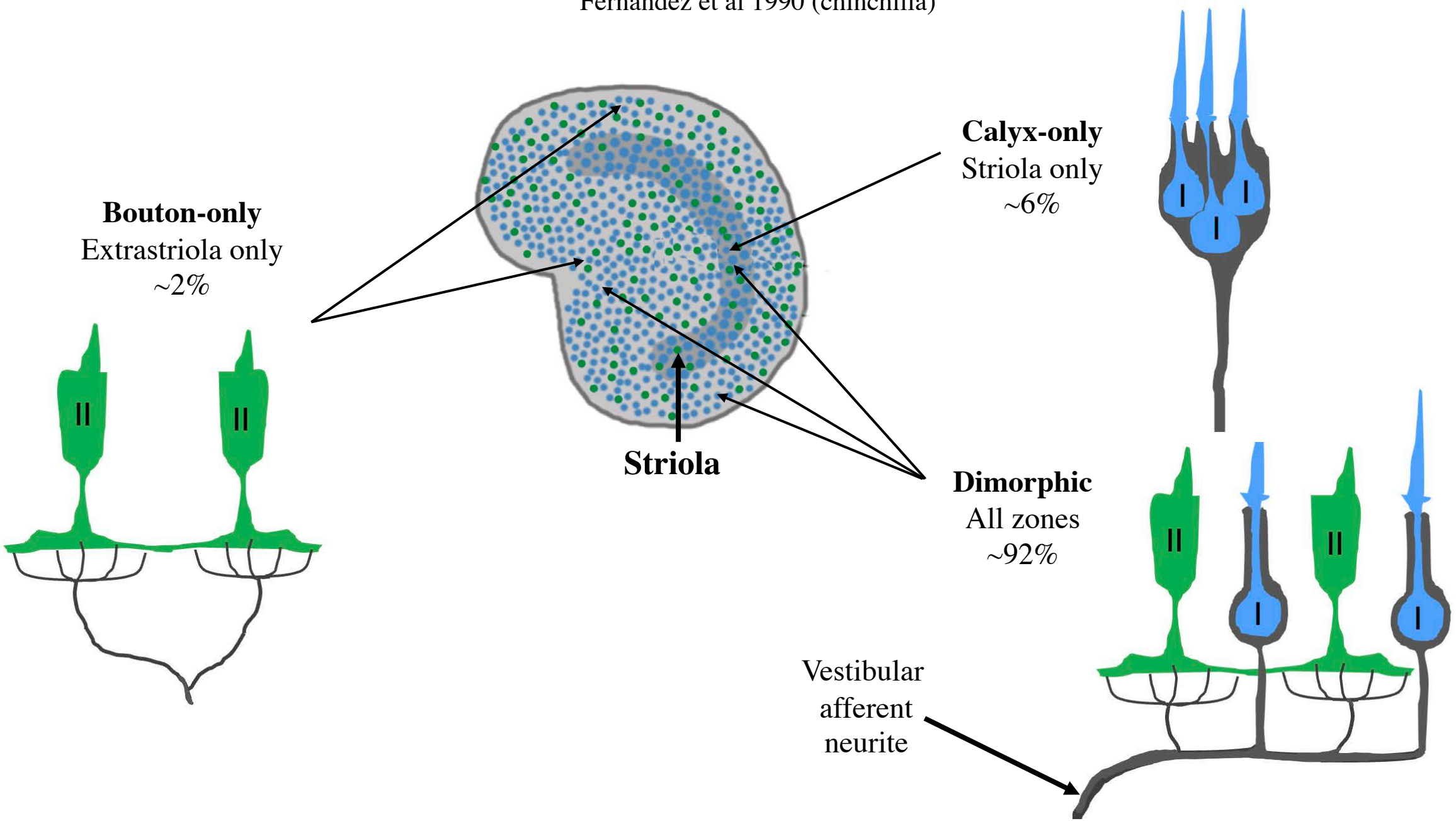
Type I hair cells are better suited to sense fast head motions

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

ePhysiology — **Hair cell membrane conductances: gK_{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

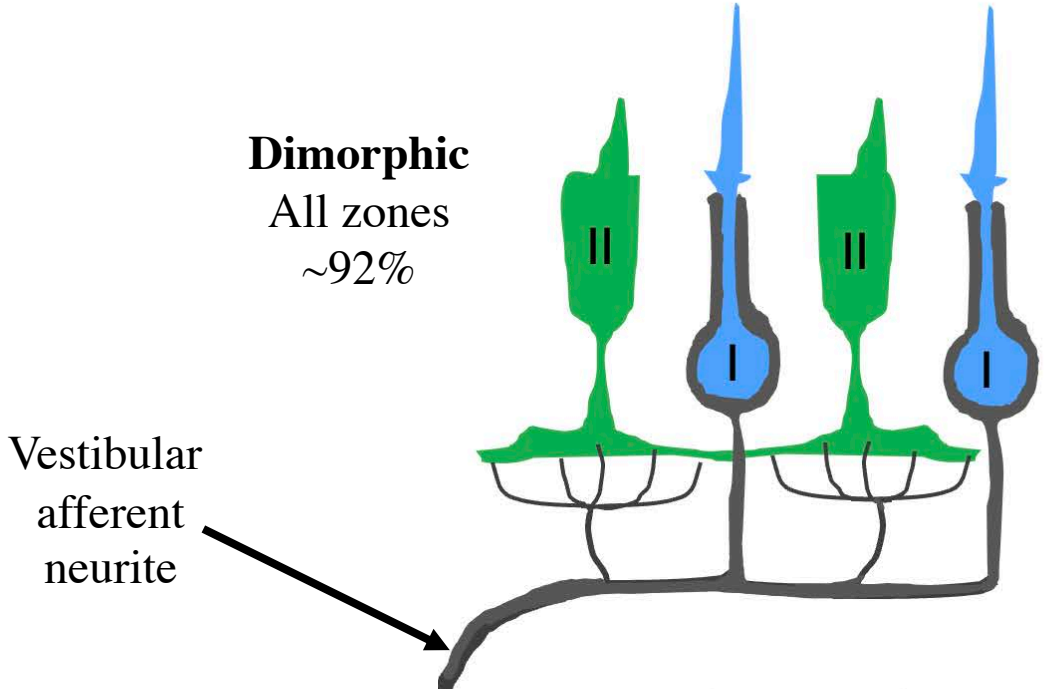
Fernandez et al 1990 (chinchilla)



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!

Dimorphic
All zones
~92%



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

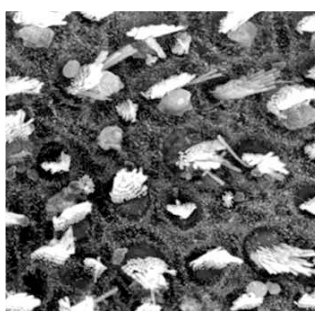
It is likely that new hair cells must:

- 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

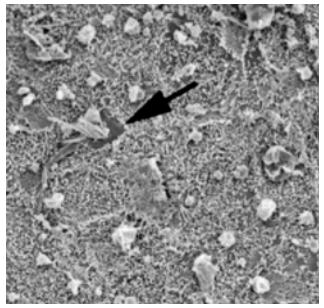
It is likely that both type I and II hair cells will need to be replaced

Identify ways to overcome the shortcomings of our natural regenerative ability to promote full functional recovery

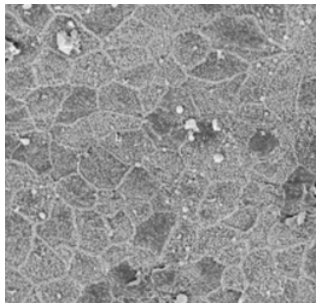
Anatomical evidence suggests humans can regenerate some vestibular hair cells



Young adult guinea pig

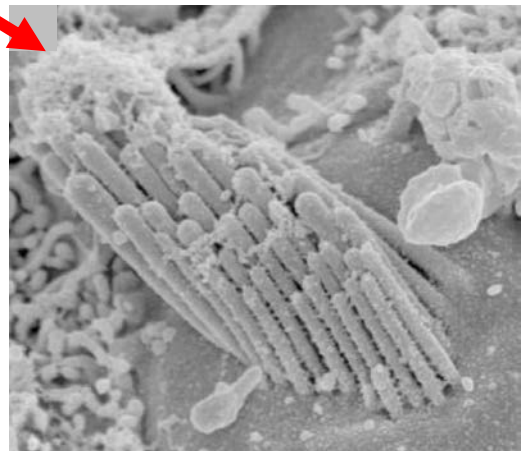


80 year-old woman

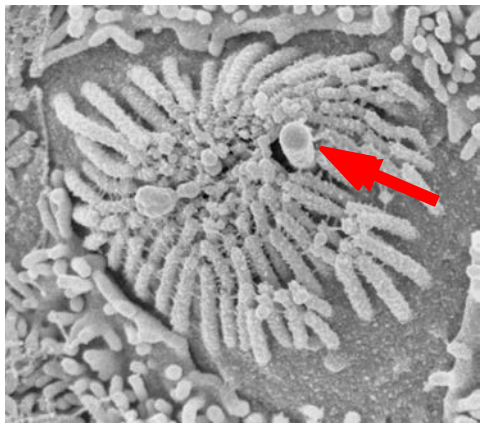


65 year-old man

Human vestibular hair cells



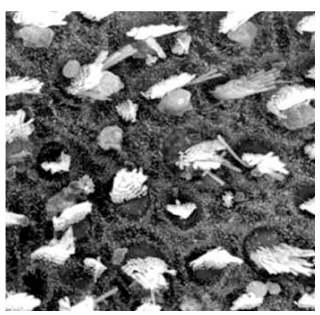
Normal



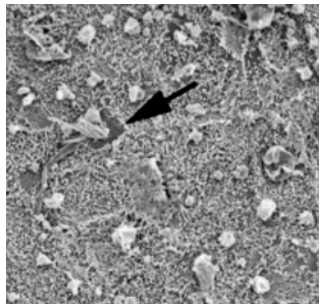
Regenerated?

Taylor et al. 2015, 2018

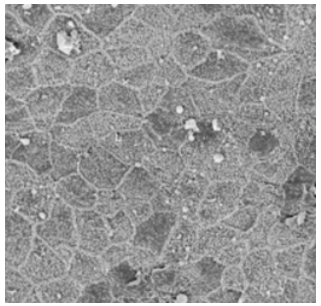
Anatomical evidence suggests humans can regenerate some vestibular hair cells



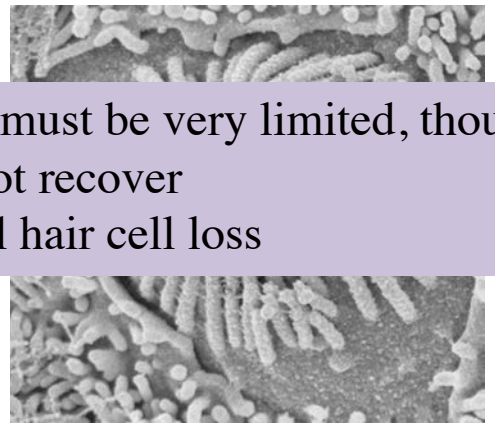
Young adult
guinea pig



80 year-old
woman



65 year-old
man



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

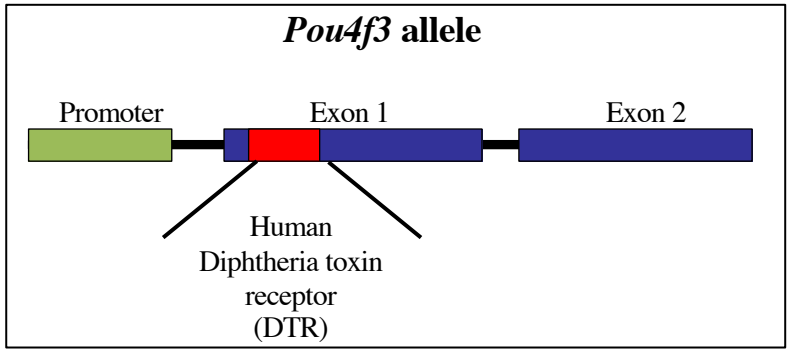
Normal

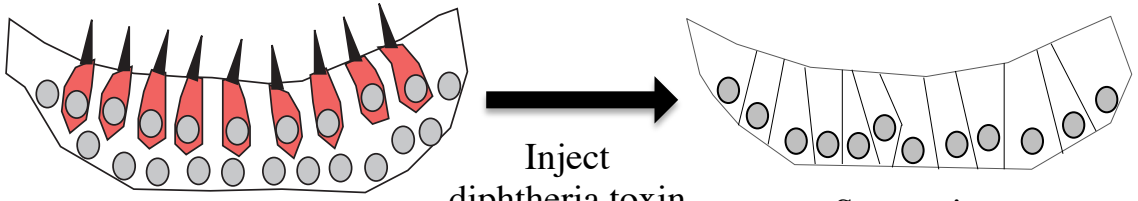
Regenerated?

Taylor et al. 2015, 2018

Mice are valuable models to study vestibular hair cell regeneration

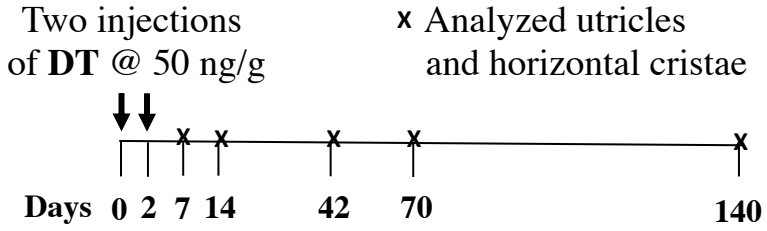
***Pou4f3*^{DTR} *(DTR)* mouse -> selective ablation of hair cells**





Experimental design

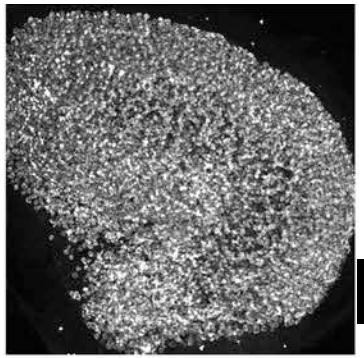
Adult (6-9 week old) mice - wildtype (WT) or *DTR* heterozygotes



Golub et al. 2012

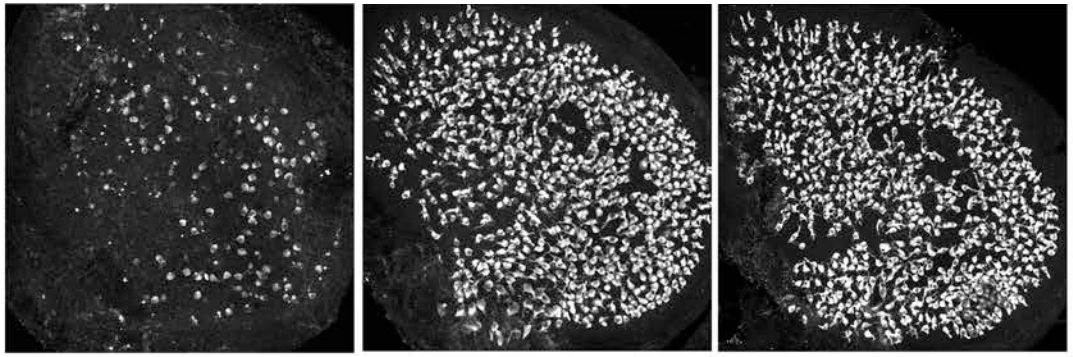
Many vestibular hair cells are naturally regenerated in adult mice

Whole utricle, top down view



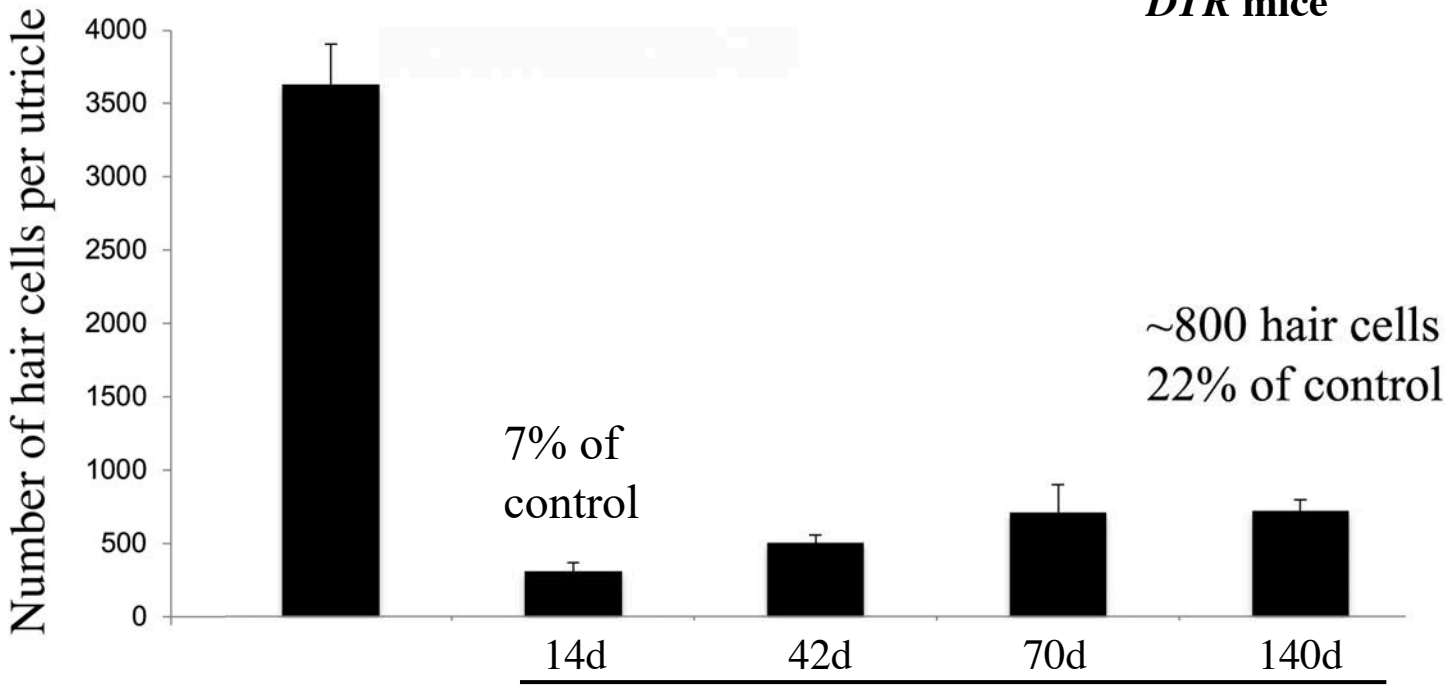
Myosin 7a

Undamaged control



14d post-DT 70d post-DT 140d post-DT

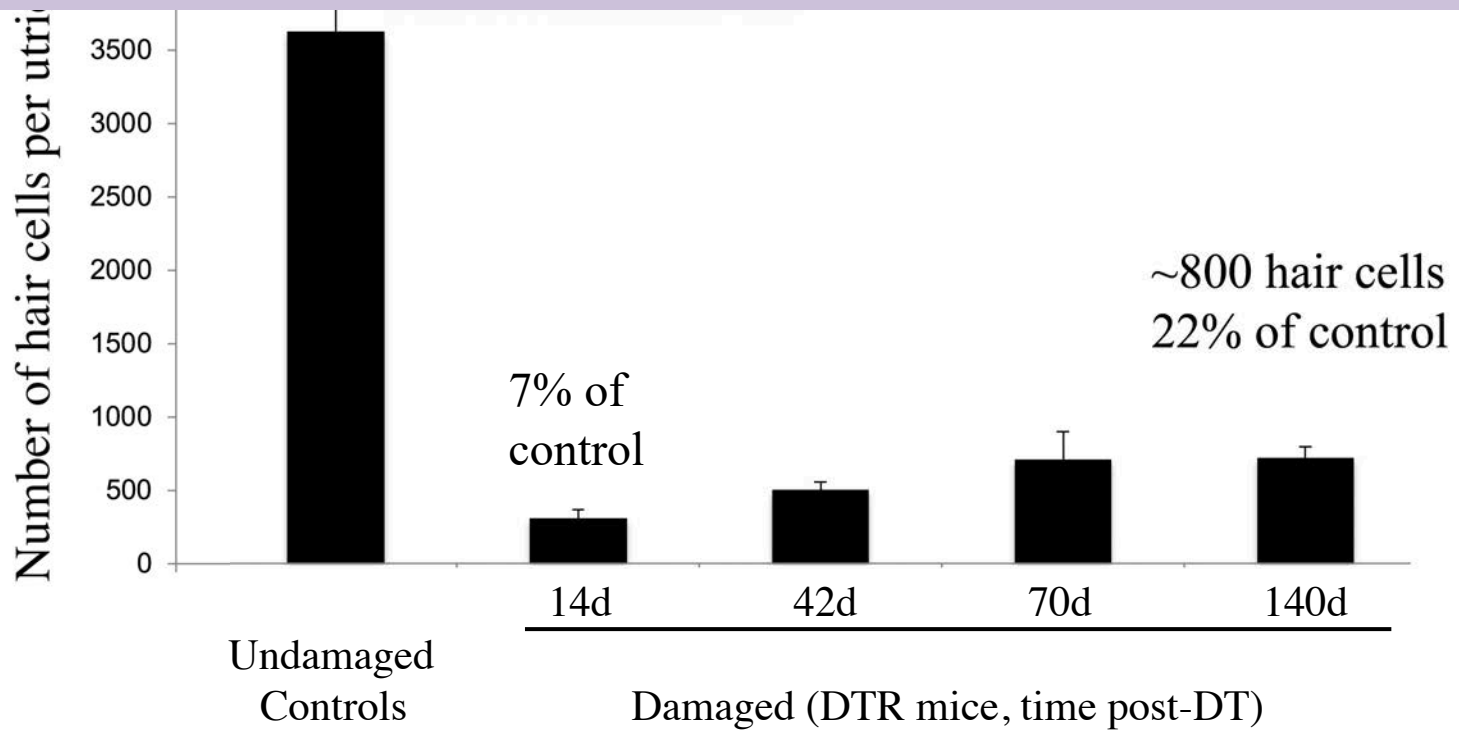
***DTR* mice**



Golub et al. 2012

Many vestibular hair cells are naturally regenerated in adult mice

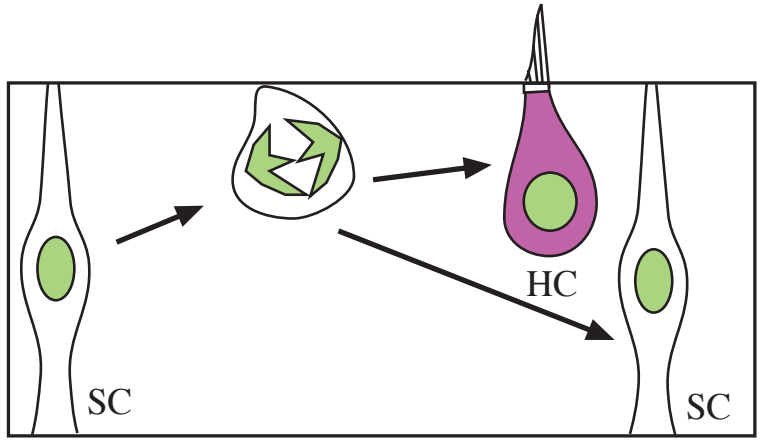
The other vestibular organs have a similar degree of damage and regeneration



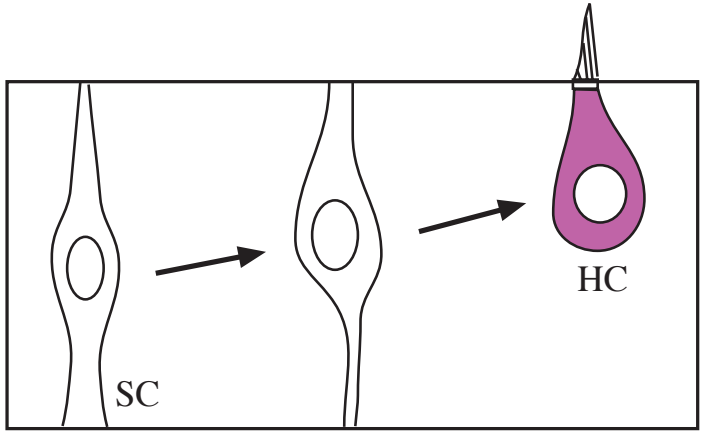
Golub et al. 2012

In rodents, new hair cells are formed by supporting cells via a non-mitotic mechanism

Mitotic regeneration



Non-Mitotic regeneration
(direct transdifferentiation)



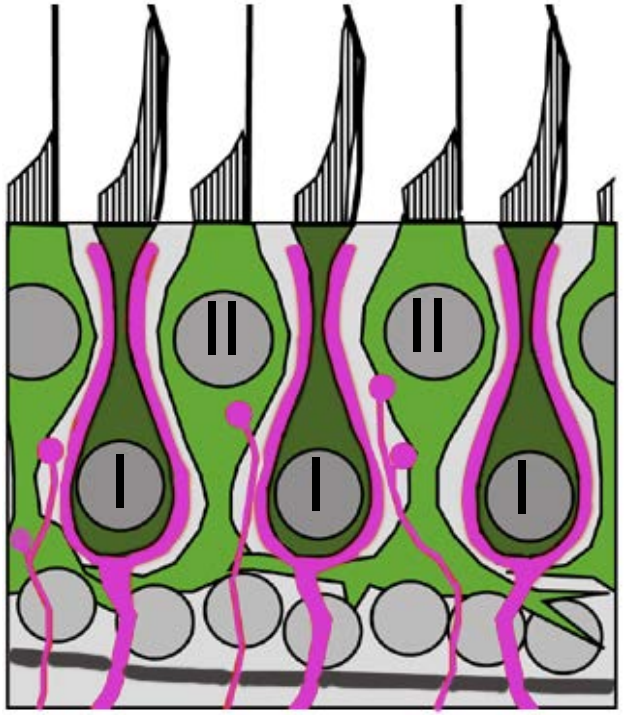
Birds and amphibians

Mammals

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

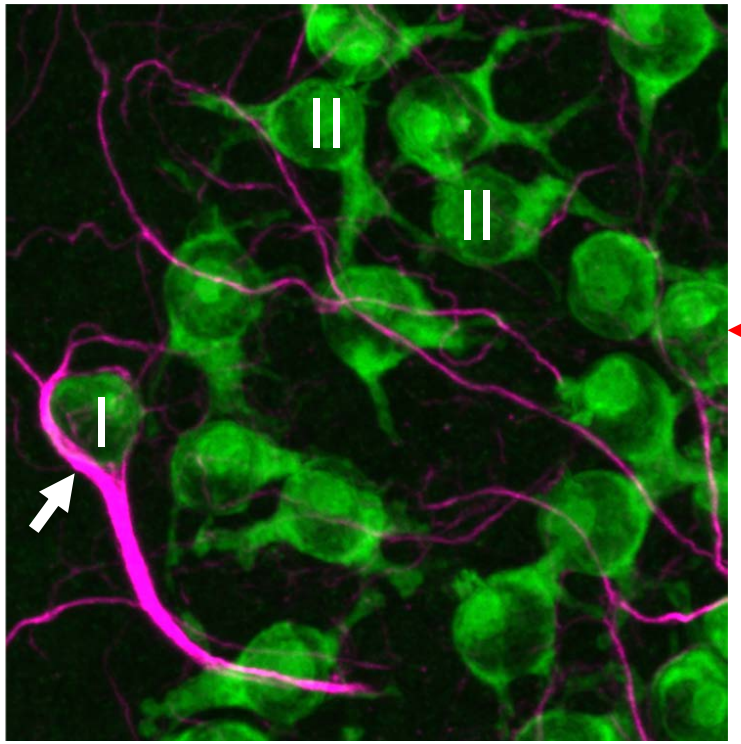
Regenerated hair cells are type II-like: Lack afferent calyx ending

Normal mice
(cross section)

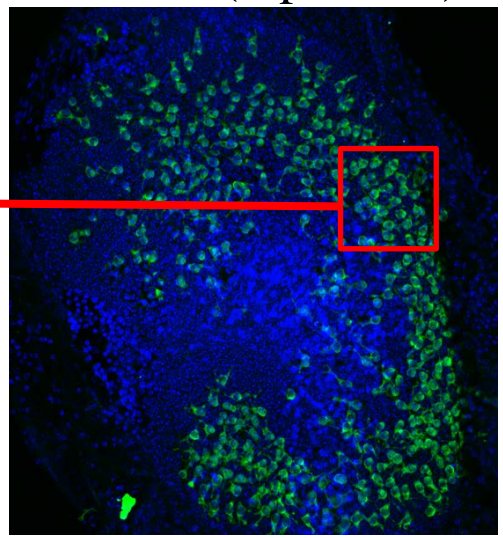


Myosin – hair cell
Neurofilament – afferent nerves

DTR mouse, 90 days post-DT
(top-down view)



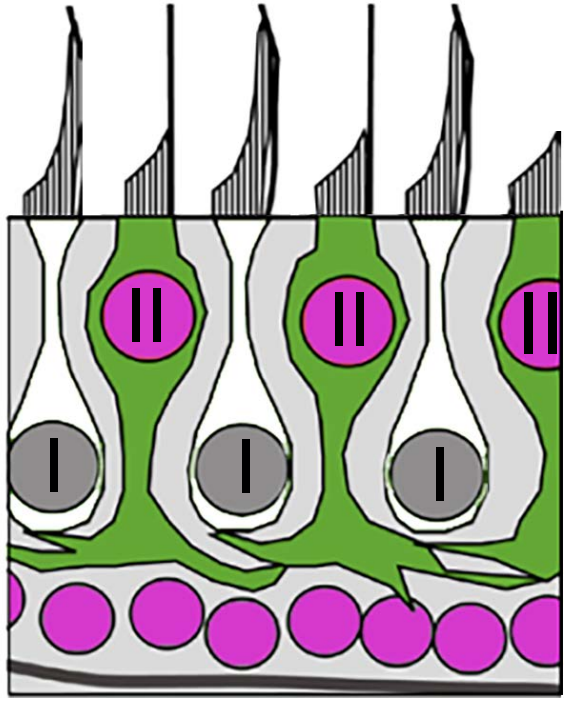
Utricle (top-down)



Golub et al. 2012

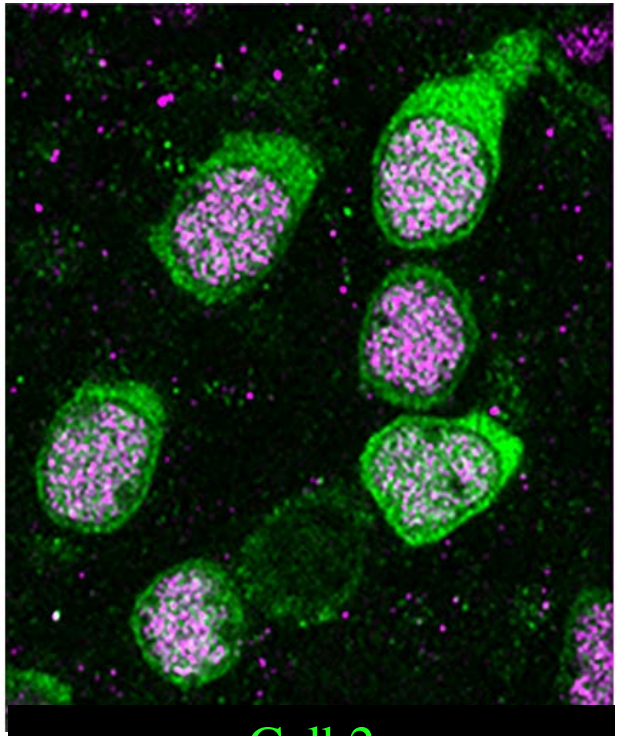
Regenerated hair cells are type II-like: Express the markers Calb2 and Sox2

Normal mice



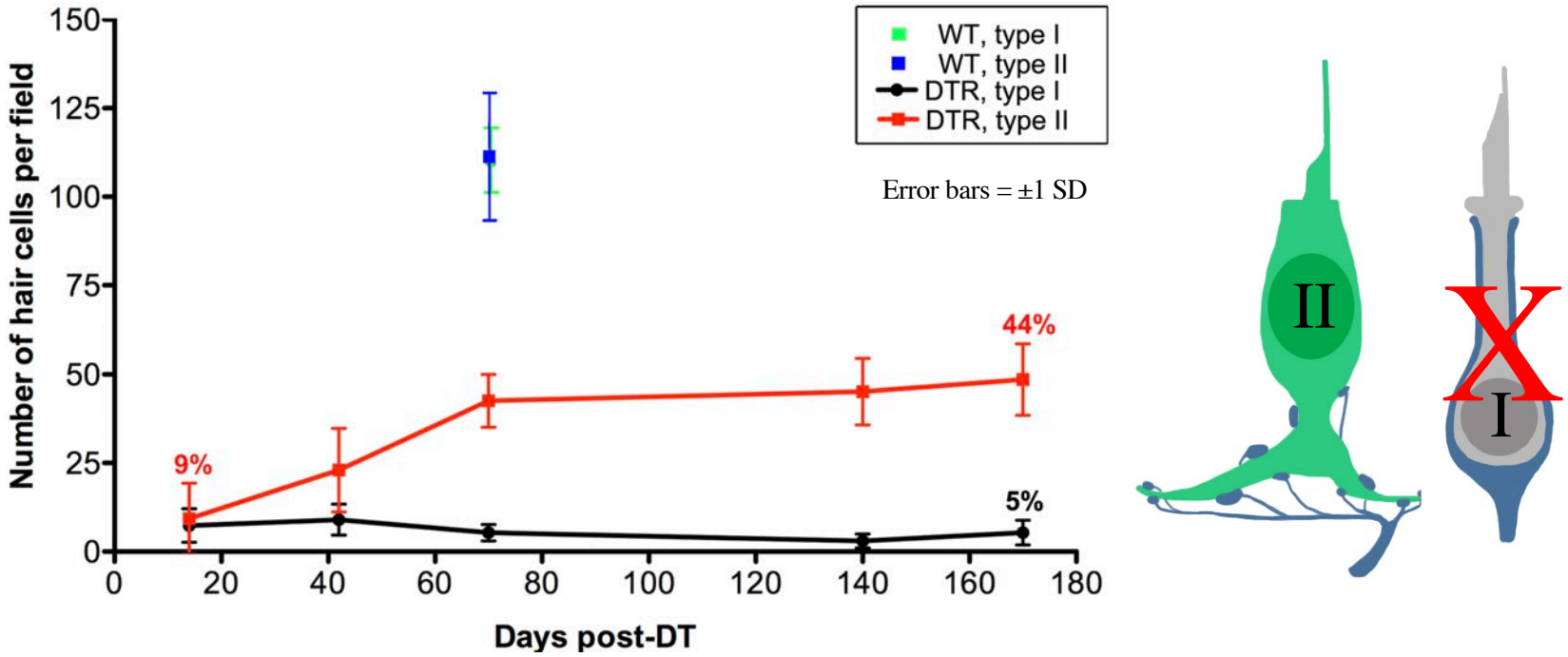
Calb2
Sox2

DTR mouse, 21 days post-DT

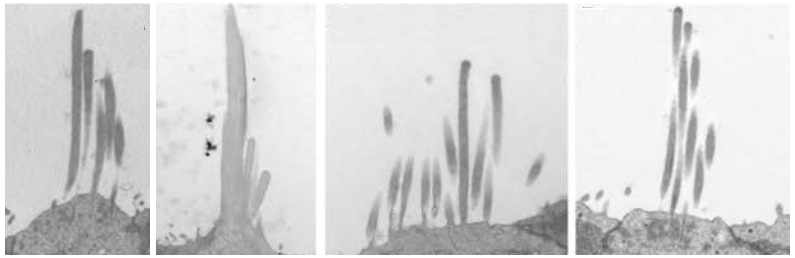


Calb2
Sox2

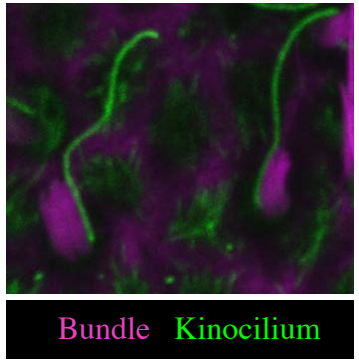
All regenerated hair cells are type II-like (utricle)

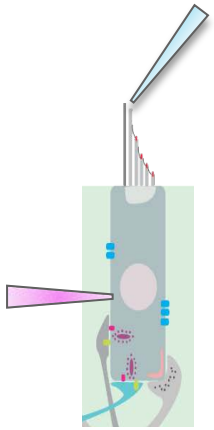


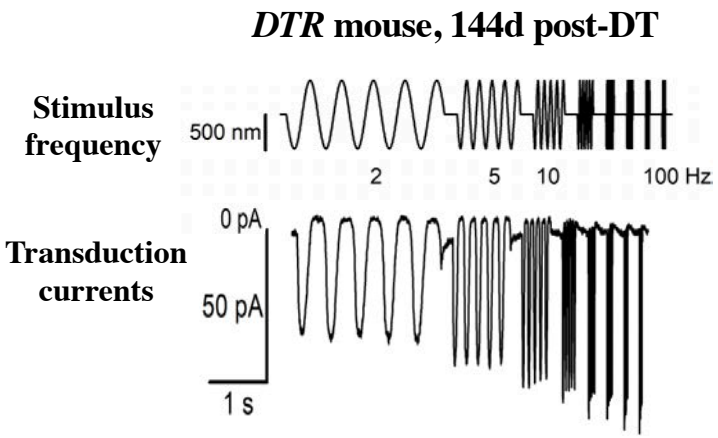
New hair cells acquire appropriate bundle structure, and they mechanotransduce (utricle)

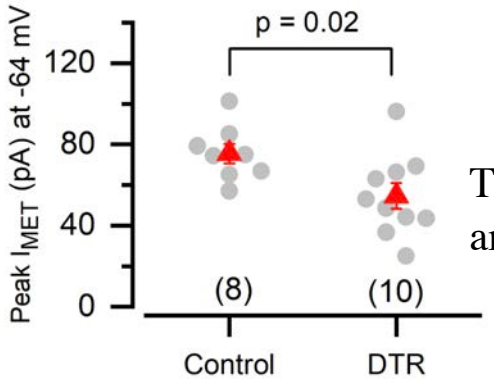


- Stereocilia height and diameter are within normal range
- Bundle asymmetry and orientations are normal
- Otoconial membrane and otoconia are intact







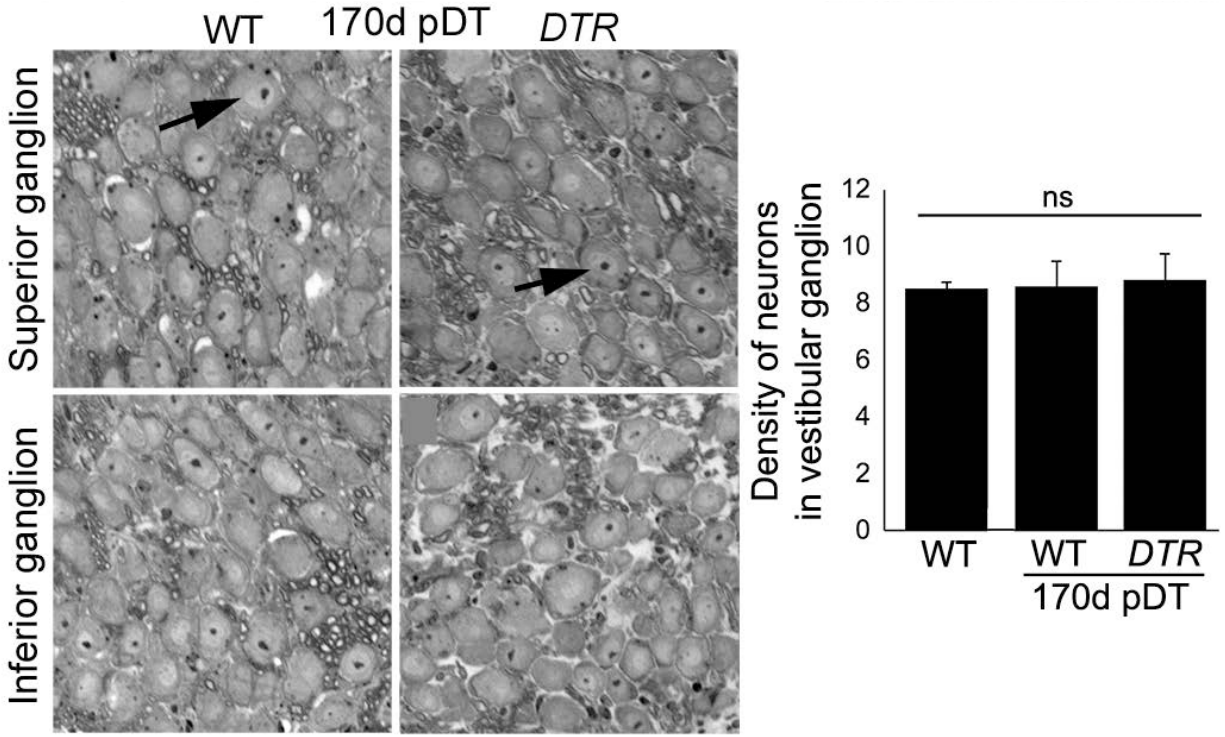


Transduction currents are smaller in regenerated hair cells

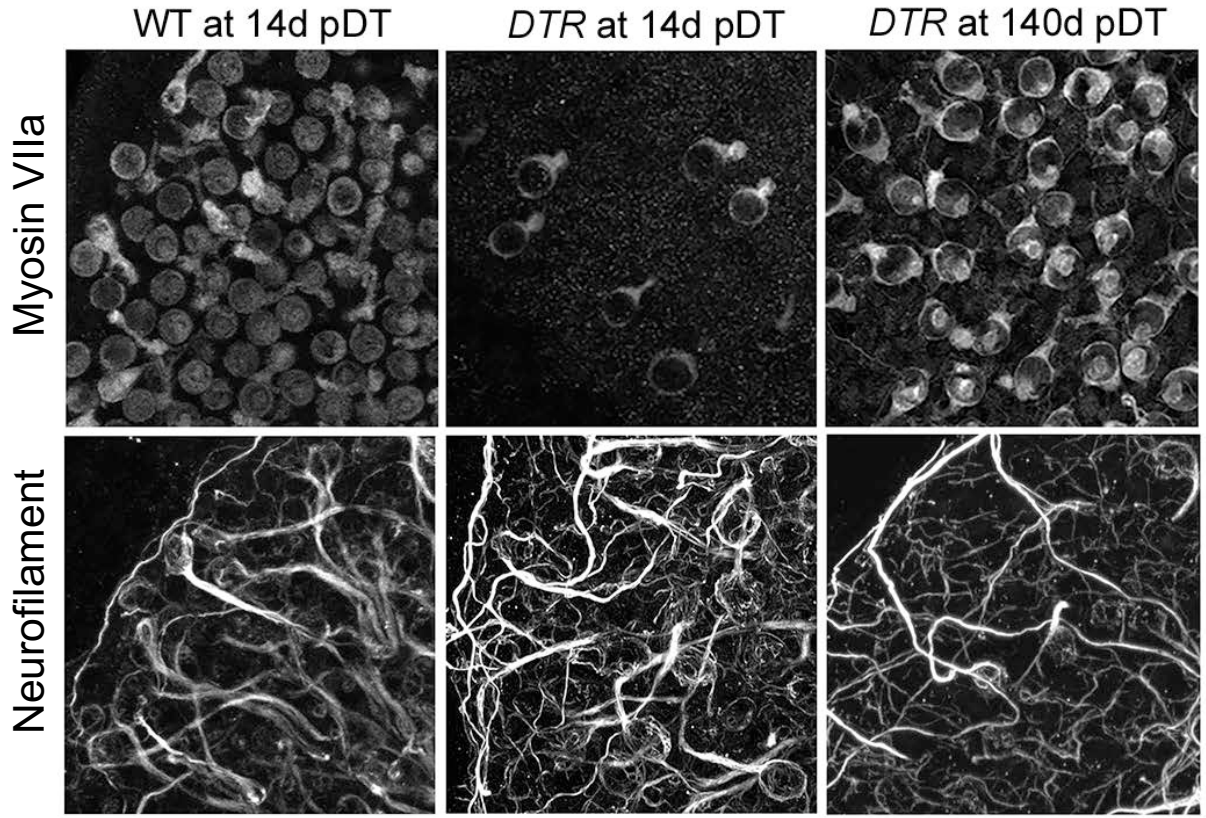
González Garrido et al. 2021

Vestibular ganglion neurons survive after hair cell destruction, and neurites remain in/return to the sensory epithelia

Vestibular ganglion

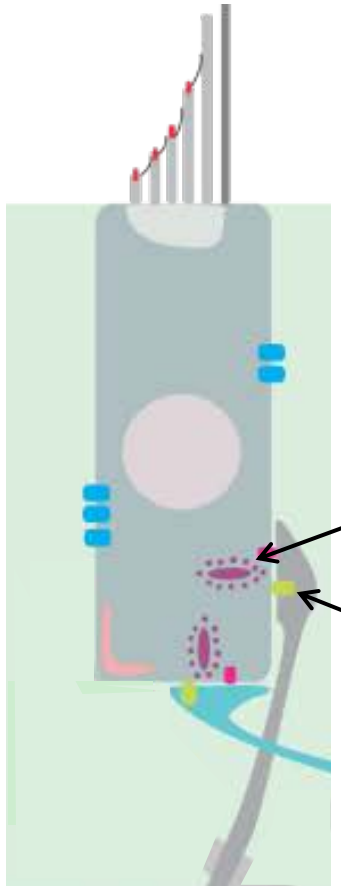


Utricle



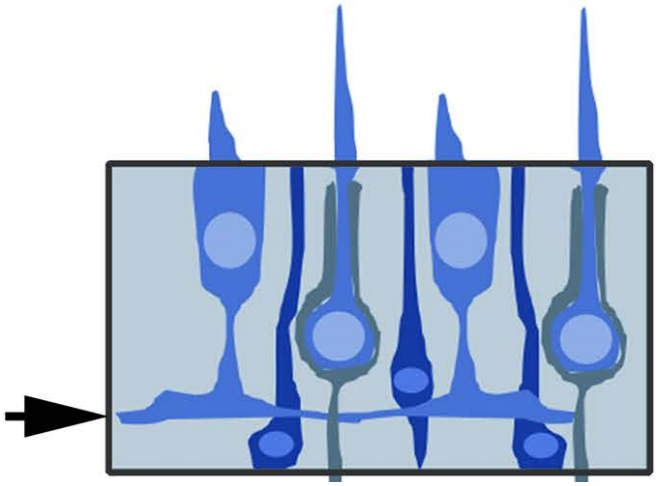
Jáuregui et al, in preparation

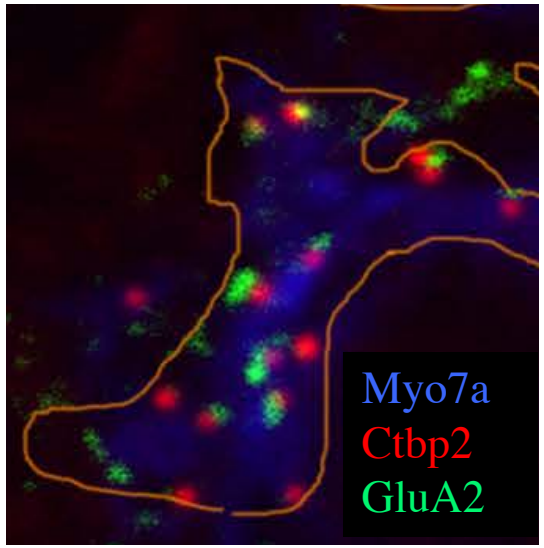
Regenerated hair cells make ribbon synapses with vestibular afferents



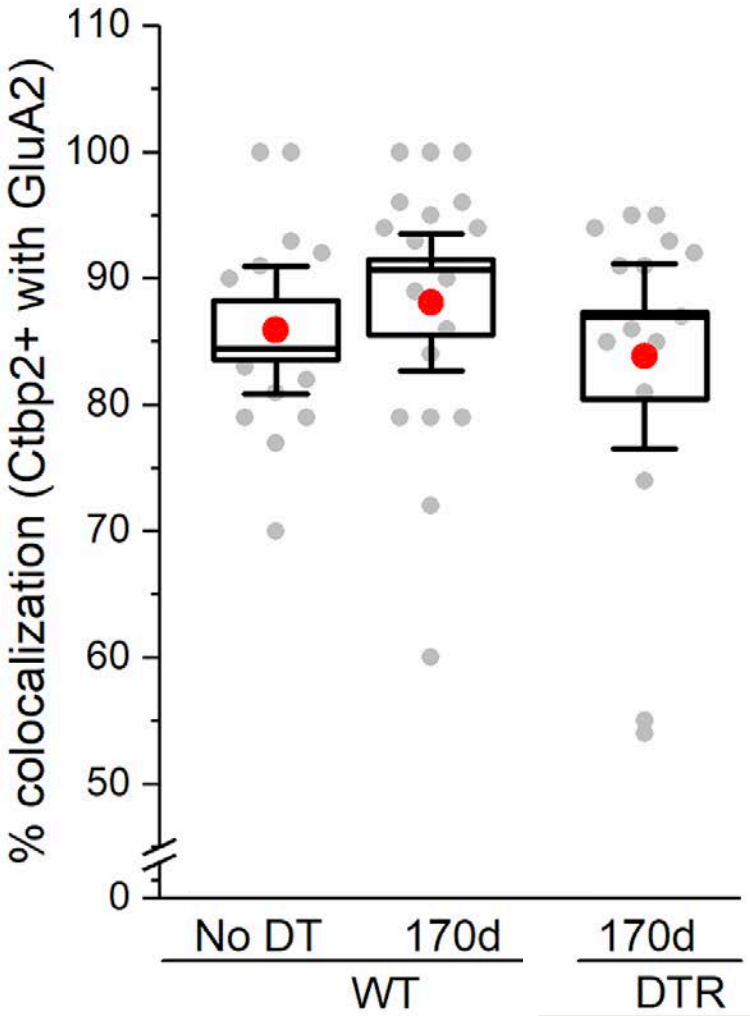
Pre-synaptic ribbons **Ctbp2**

GluA2 Glutamate receptors in bouton





% of Ctbp2+ particles that co-localize with GluA2



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