Structural and Functional Adaptation of the Vestibular Otolith to Altered Gravity from Microgravity to Hypergravity

Richard Boyle

NASA Ames Research Center, California

Chief Editor, Section “Environmental, aviation and space physiology” *Frontiers in Physiology*

Fish: electrophysiology/synaptic organization

<u>µg</u>

<16-day: STS-90, -95

<u>Hyper-g: 3 (2.24 resultant) g</u>

1-32 day/readaptation

Mice: otoconia structure

<u>µg</u>

~13-day: STS-133, -135

90-day MDS: STS-128, -129

<u>Hyper-g: 2 (1.41 resultant) g</u>

90-day MDS: Osaka

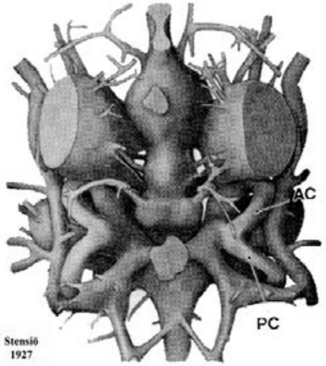
General Concepts Motivating the Study

1) Gravity has remained constant during animal evolution.

The inner ear utricular organ of the vestibular labyrinth senses the sum of inertial force due to head translation and head tilt relative to gravitational vertical. A change in gravity or orientation with respect to gravity would be expected to have a profound effect on how an organism interacts with its environment and maintain equilibrium.

General Concepts Motivating the Study

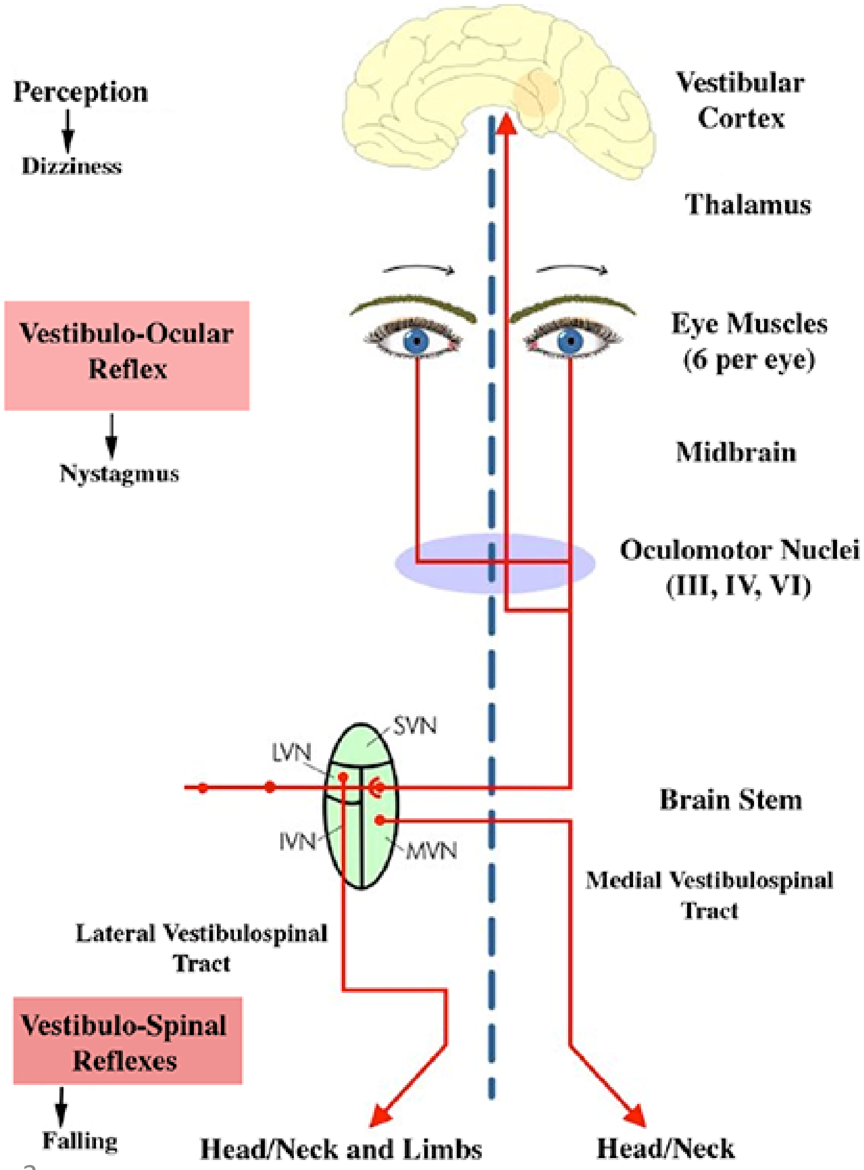
2) Pervasiveness of gravity provides the nervous system a common reference about which to optimize sensory transduction mechanisms and perception. The first vertebrate ear was principally a graviceptive statocyst more commonly found in today’s aquatic invertebrates. A highly conserved neural sensing system detecting accelerative forces has evolved in vertebrates. The neural sensory systems resolve the ambiguity of gravity and self-motion to allow healthy individuals to maintain balance and equilibrium under varying conditions commonly encountered on Earth..



Stensiö 1927

Fossil ostracodern, Kiaeraspis auchenaspididoides
Jawless vertebrate in the Devonial Period (Paleozoic) 400 million years ago

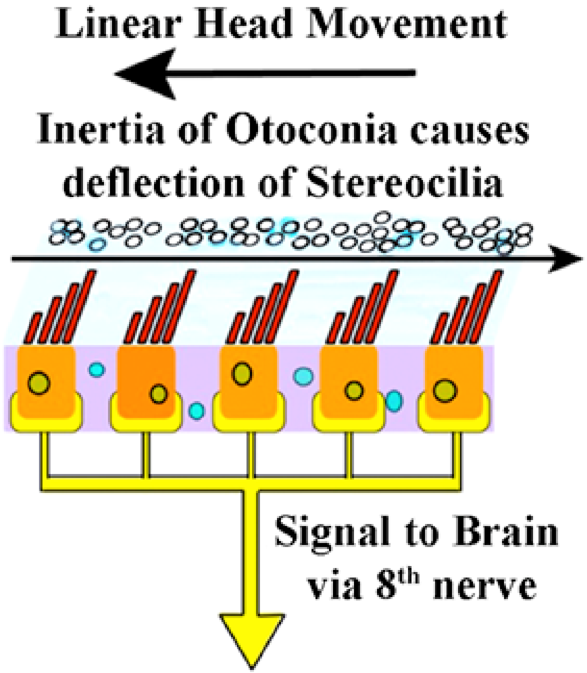
General Concepts Motivating the Study



3) Despite invariability of gravity on Earth, physiological systems *are not* and are adversely affected by trauma, disease, and aging, and thus the central internal creation of the gravito-inertial vector can be disrupted leading to vertigo, disorientation, and other clinical disorders. **Gaze, posture and locomotion** are directly controlled by the neurovestibular system and, if altered, performance along the neuraxis will be adversely affected temporarily or even lost without intervention on Earth and in space.

General Concepts Motivating the Study

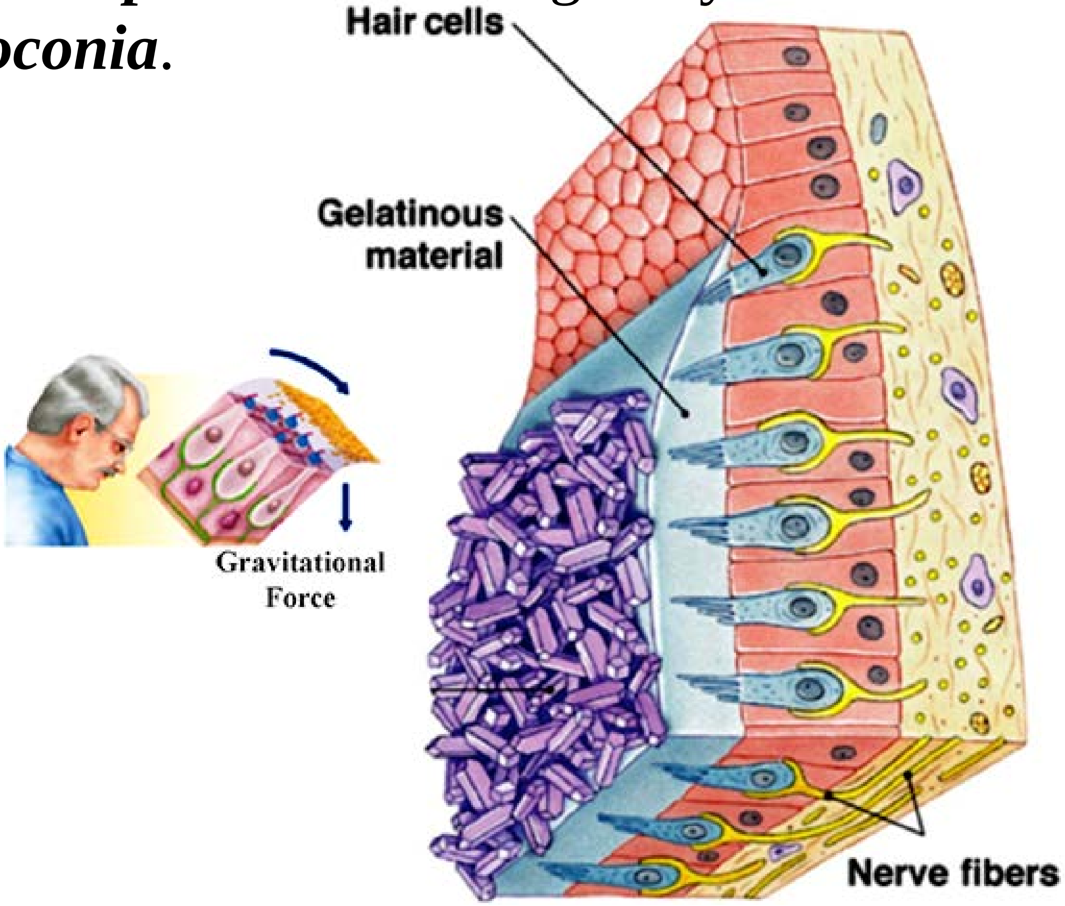
(4) Vertebrates use weight-lending structures made of $CaCO_3$ to enable the transduction of gravito-inertial accelerations into neural signals carried by the 8th cranial nerve otolith afferents to the brain. We will examine *first* the otolith ***afferent response*** to altered gravity and *second* the structural changes of the ***otoconia***.



Linear Head Movement

Inertia of Otoconia causes deflection of Stereocilia

Signal to Brain via 8th nerve



Understanding the Fundamental Mechanisms of Neurovestibular Plasticity

Electrophysiological techniques were used in oyster toadfish to evaluate the response properties of utricular otolith afferents to applied accelerations upon return to 1g following a relatively brief exposure to weightlessness aboard the STS-90 (NeuroLab, 15 days) and STS-95 (Endeavor, 8 days) missions.

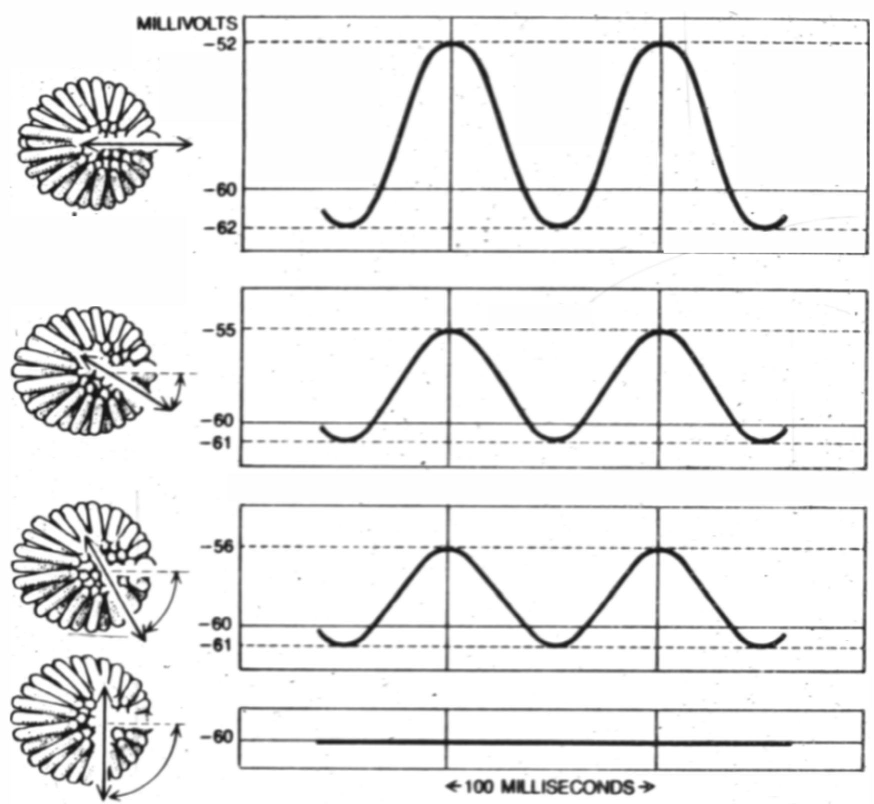
Fish Package Control Units #1 and #2 in Spacelab

Pre-flight identification record of Utricular Afferent Response to linear acceleration

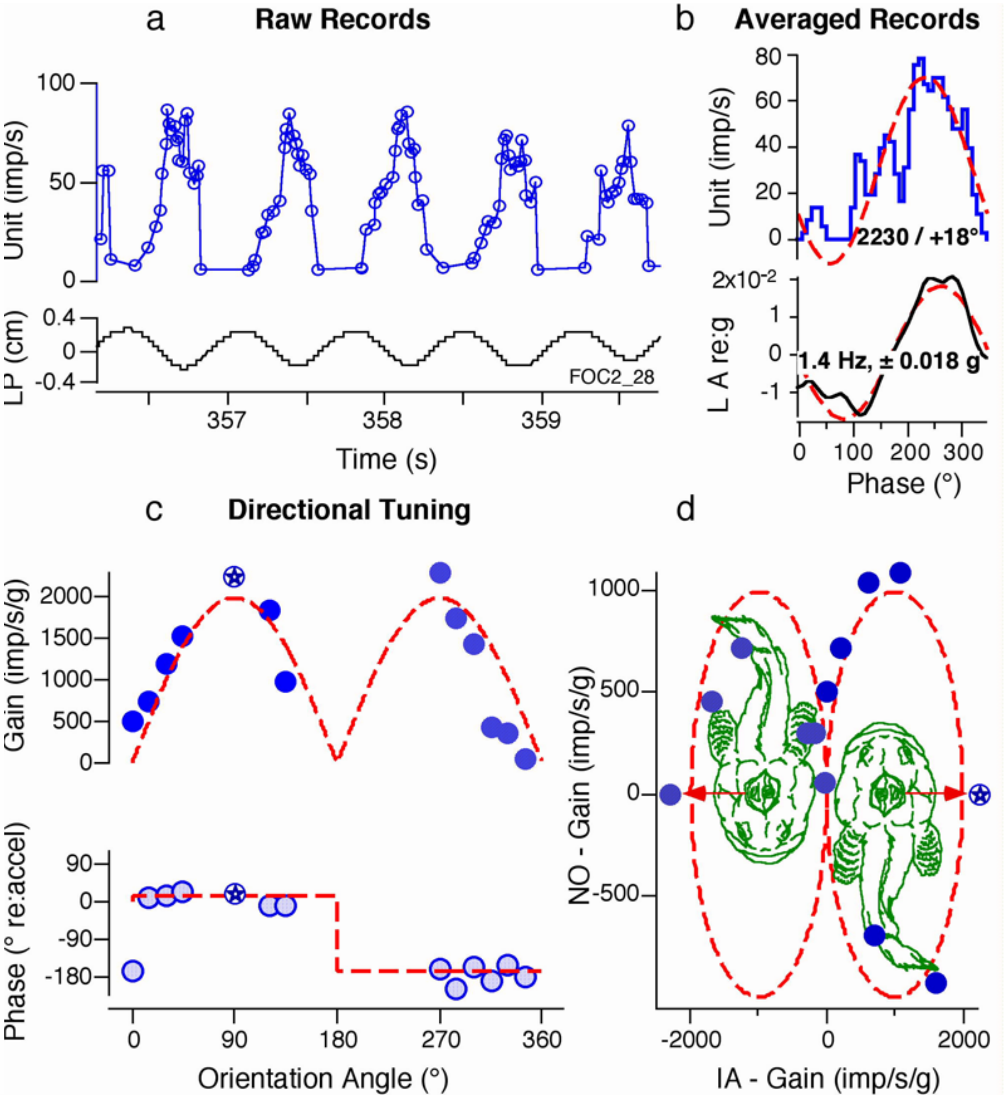
Hair Cells and Afferents are Directionally Sensitive

Hair Cell

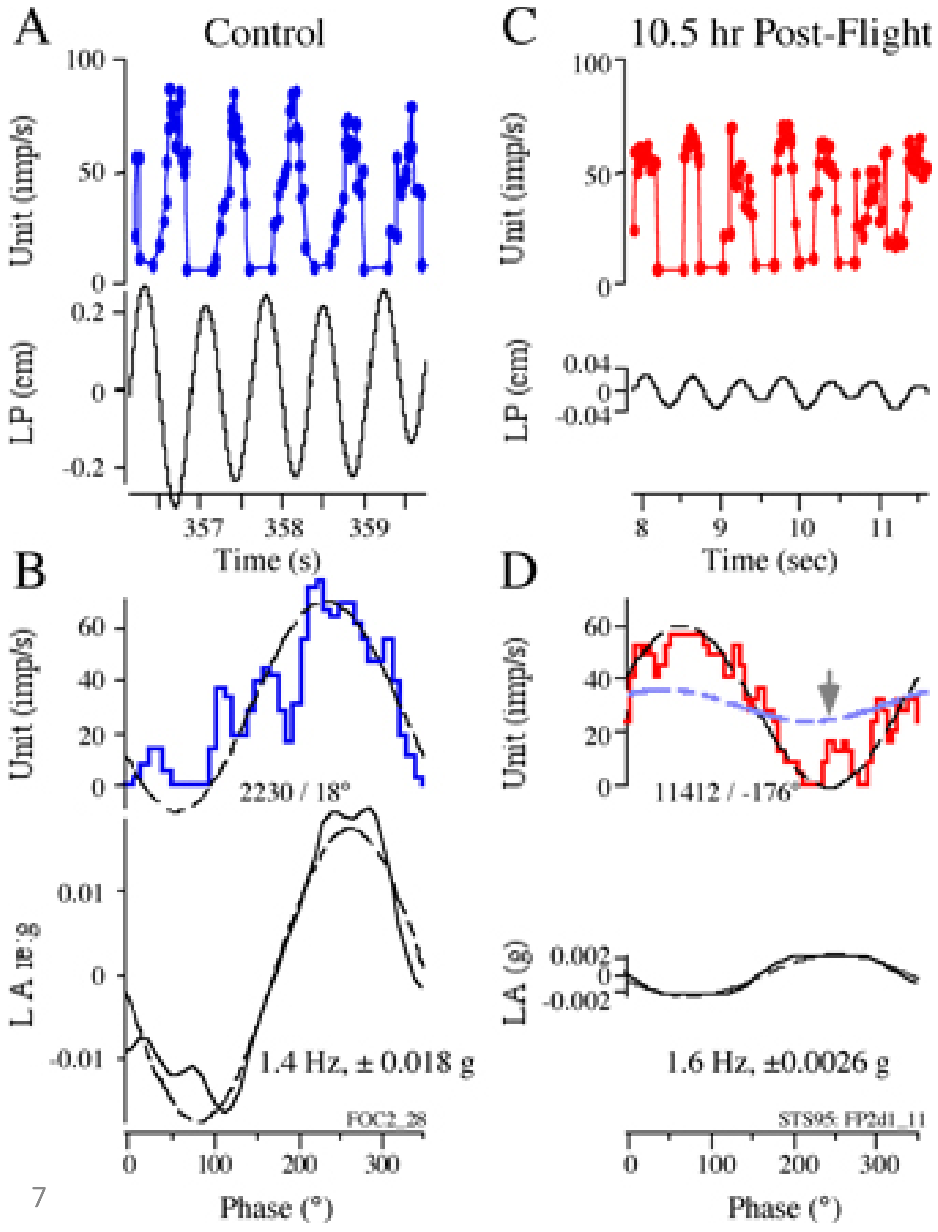
Utricular Afferent



DIRECTIONAL SENSITIVITY of the hair cell is shown in traces representing recordings made with an electrode inserted into a single receptor from the saccule of the bullfrog while its hair bundle was being moved. The figures are based on experimental records made by Sandra L. Shotwell in the author's laboratory. When the hair cell is in its resting state, its interior has an electric potential 60 millivolts lower than that of the surrounding fluid. If the hair bundle is displaced toward the kinocilium along the axis of bilateral symmetry, the potential difference decreases to −52 millivolts (*top*). If the bundle is displaced away from the kinocilium along the same axis, the difference increases to −62 millivolts. As the direction of displacement diverges from the axis of symmetry the size of the response decreases (*middle panels*). The cell does not respond to displacements along the axis perpendicular to that of symmetry (*bottom*).



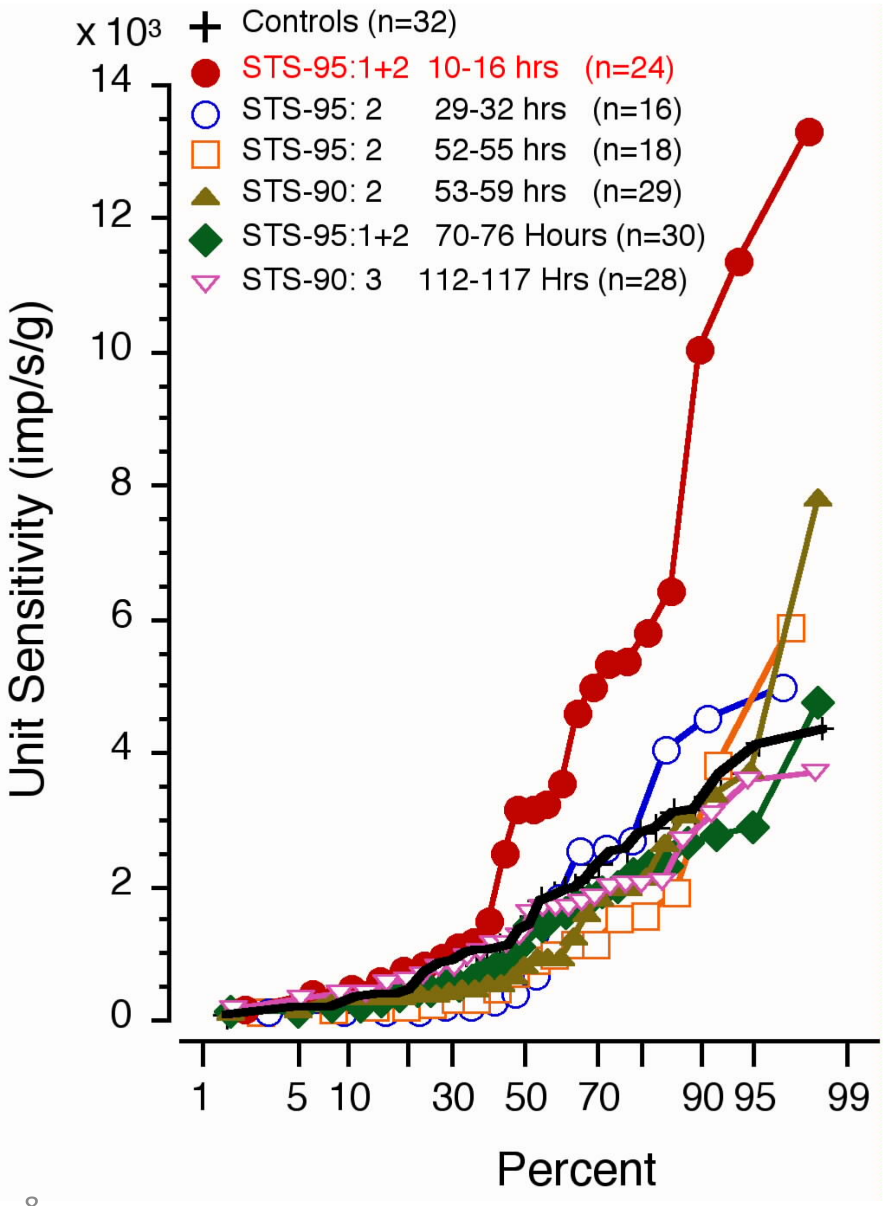
Influence of reduced gravity on the vestibular system in vertebrates



Control (A,B) and postflight (C,D) responses of utricular afferents to inertial accelerations. A,C: Maximum sensitivity (Smax) for cycles at the afferents' preferred orientation. B,D: Averaged response and stimulus (LA re:g). Ordinates in each panel are scaled equally to illustrate the primary finding: ***post-flight afferents recorded shortly after return to Earth exhibit a profound hypersensitivity to translational acceleration.***

The control response of the afferent shown in A and B is modeled as the dashed curve marked by the arrowhead in the histogram of D.

Influence of Reduced Gravity on the Afferent Sensitivity



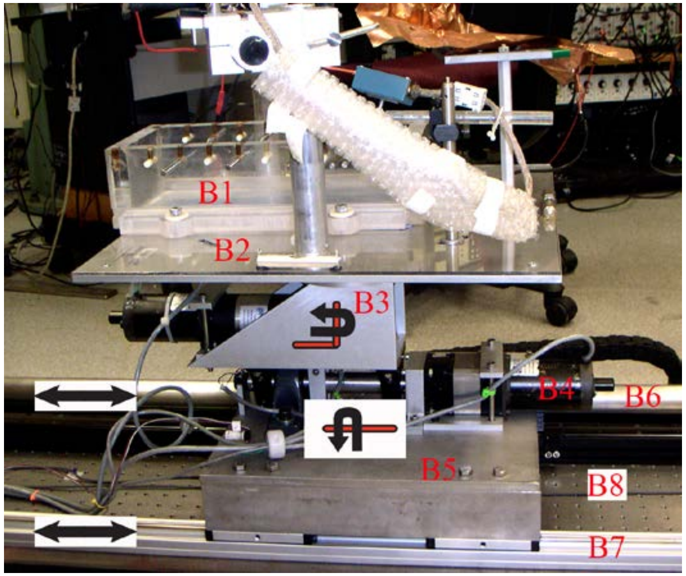
Post-flight Afferent Hypersensitivity

Unit sensitivity (in imp/s/g) of individual responses is plotted as a function of percent within each designated group. Groups are formed with respect to time post-flight of STS-90 and STS-95 Orbiters. Note that ~60% of the afferents recorded in the first session (red filled circles) had a significant increase in sensitivity above control (black line). ***Within the first day after landing, the magnitude of response was on average three-times greater than controls.***

From: Boyle R, Mensinger AF, Yoshida K, Usui S, Intravaia A, Tricas T, and Highstein SM. (2001) Neural readaptation to 1G following return from space. *J. Neurophysiol.* 86: 2118-2122.

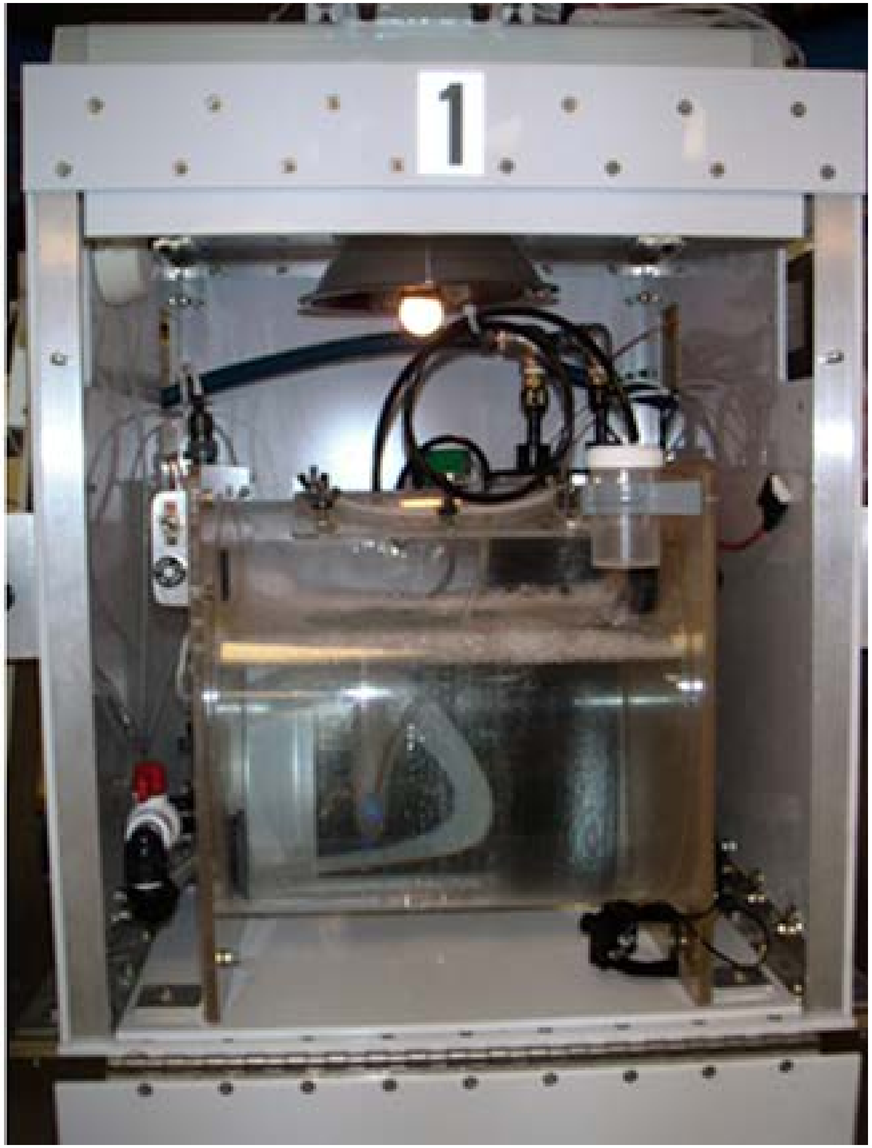
Influence of Increased Gravity and Duration of Exposure on the Vestibular System

The **Hyper-g** study is based on the following hypothesis: gravito-inertial sensitivity of otolith afferents is altered as a result of the animal's exposure to hyper-g conditions well as μg. It is reasoned that the transition from 1g to hyper-g will decrease afferent sensitivity (impulses per sec/g), and the transition from hyper-g back to 1g will increase it (back to normal levels). *Thus, the transition from hyper-g to normal gravity may serve as an imitator of neurovestibular sequelae to μg.*



Testing Apparatus: servo-controlled using WaveMetrics Igor scripts to Galil controller to drive linear acceleration (1.5m), yaw rotation (static displacement and sinusoids), and tilt (pitch to roll) motion profiles. Because of the high sensitivity and to avoid impulse rate saturation, a common linear acceleration test is ±0.03-0.05g at 1-2 Hz.

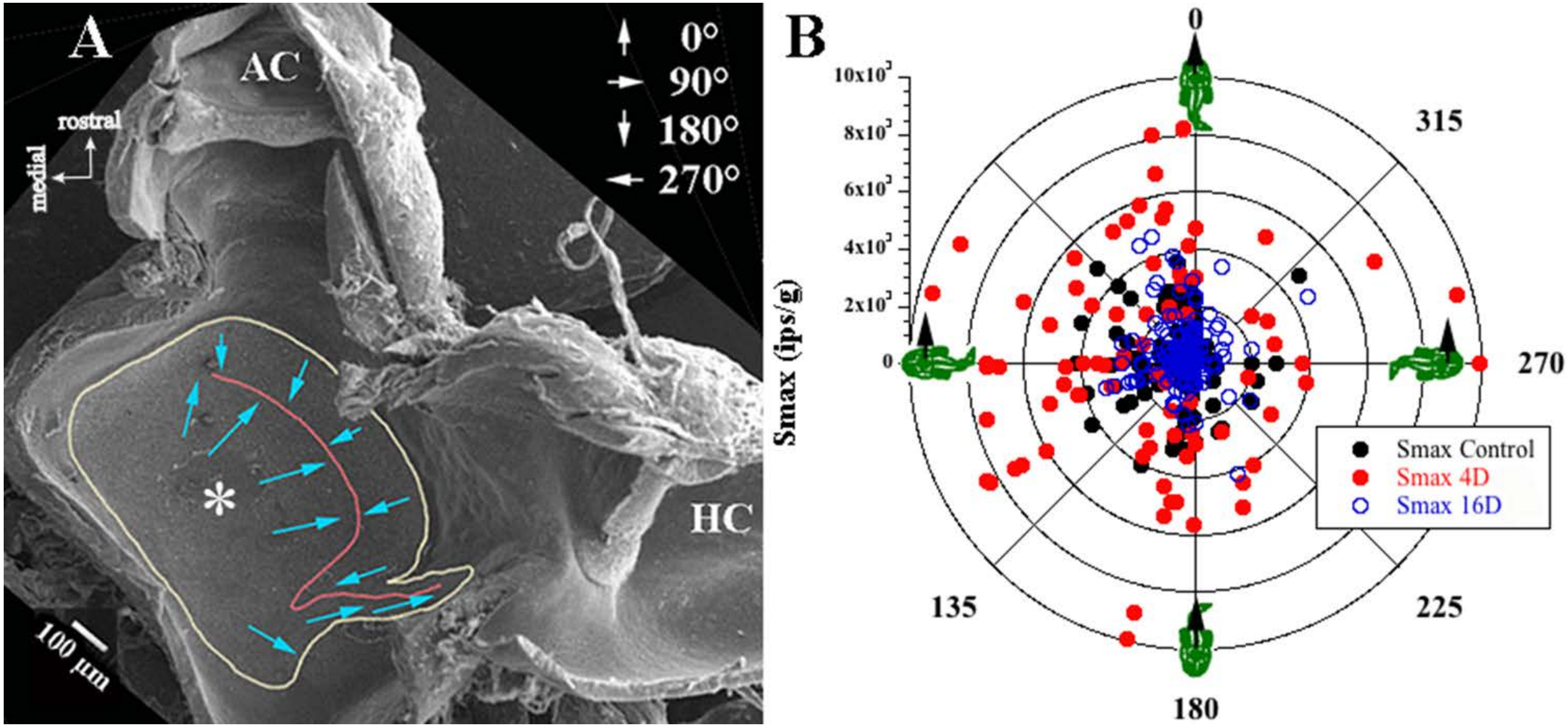
Increased Gravity achieved using the planned ISS centrifuge at Ames Research Center



Radius: 1.22m Payload: 87 kg/habitat

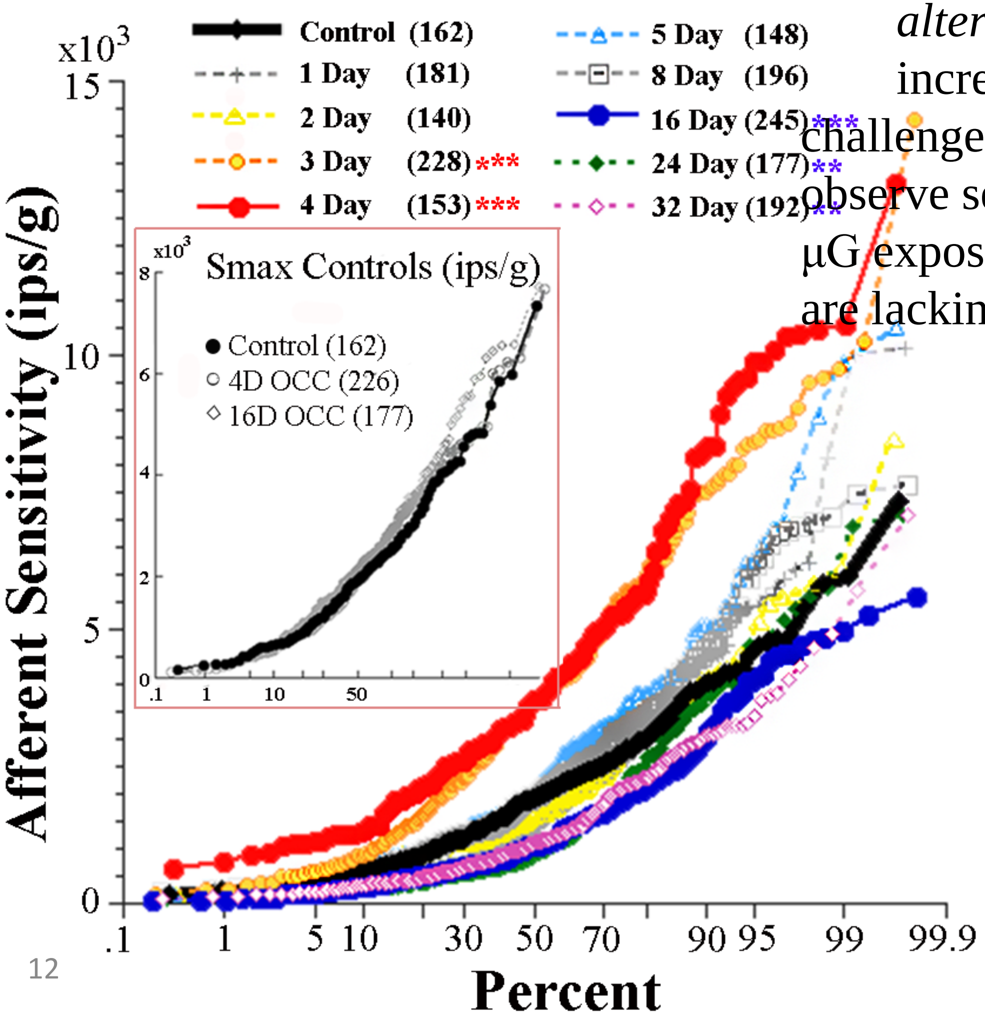
Afferent Directional Selectivity

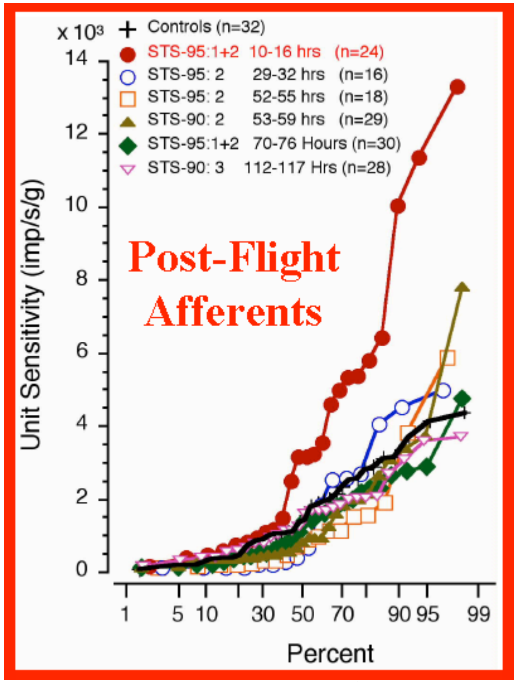
Sampling bias consideration. (A) SEM micrograph of left utricular macula flipped 180 to correspond to right utricle for comparison with data in **B**. View from above utricular macula -yellow, reversal line in – red (Boyle et al. (2018) **(B)** Afferent responses (ips/g) are plotted in a polar graph as a function of head angle (°) at Smax for control fish and for fish exposed to 2.24 g for 4 or 16 days. Head angle at Smax of recorded responses was consistent for all fish, *indicating no evidence of sampling bias from one region to another or between experimental groups.*



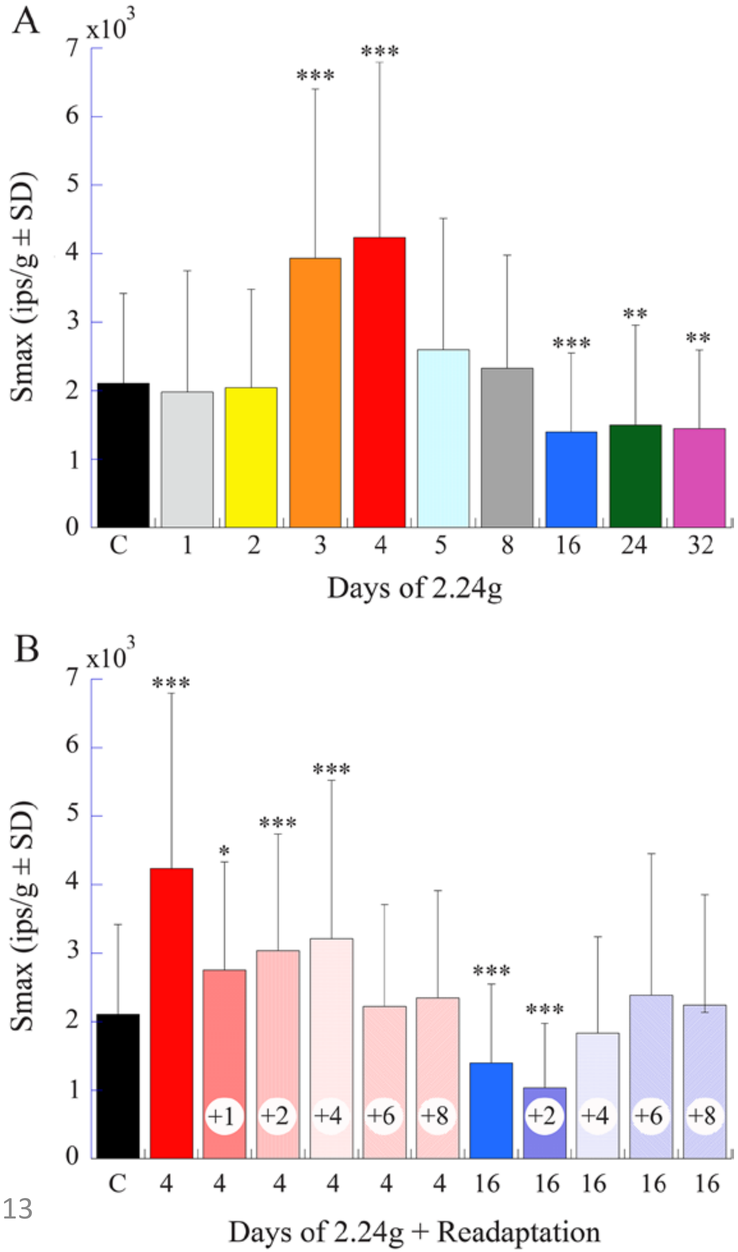
Influence of Increased Gravity and Duration of Exposure on Afferent Sensitivity

Mean Smax of control (black) and 2.24g exposed (colored symbols) afferents translation in percent plot. *Significant elevation at 3- and 4-day was observed*. The elevation was followed after 5 and 8 days of normal Smax and then by a significant decrease at 16-, 24-, and 32-day. Number of afferents recorded in each group is given in parentheses. *It is possible that the altered gravity (AG) initiates first* a large gain increase in response to a challenge, any AG challenge. In this scenario we would expect to observe sensitivity changes in early stages of µG exposure as well. Regrettably, those data are lacking.



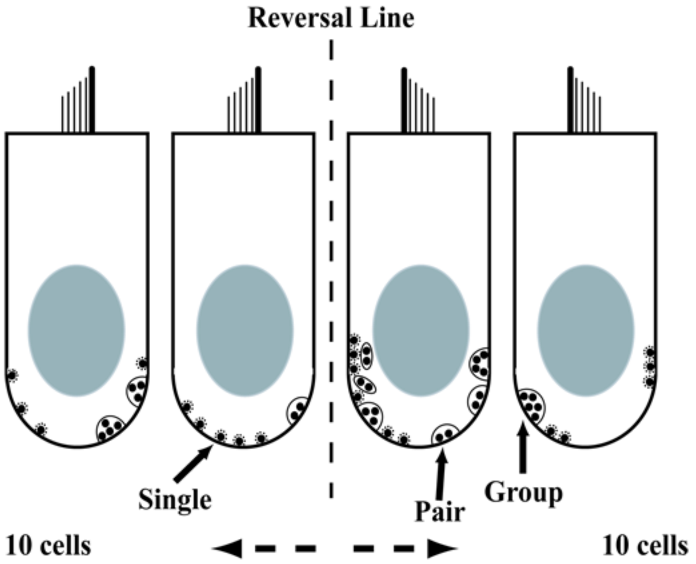


Afferent Smax as a function of days of exposure to 2.24 g and readaptation after return to normal 1 g.

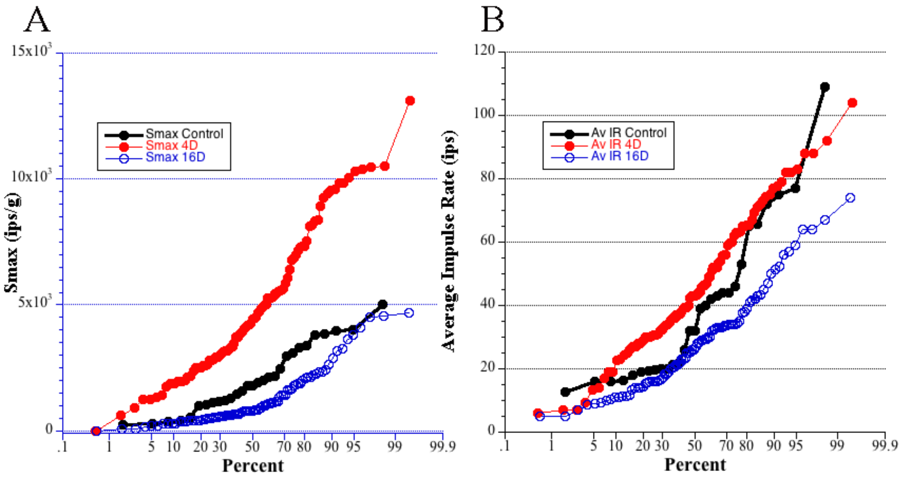


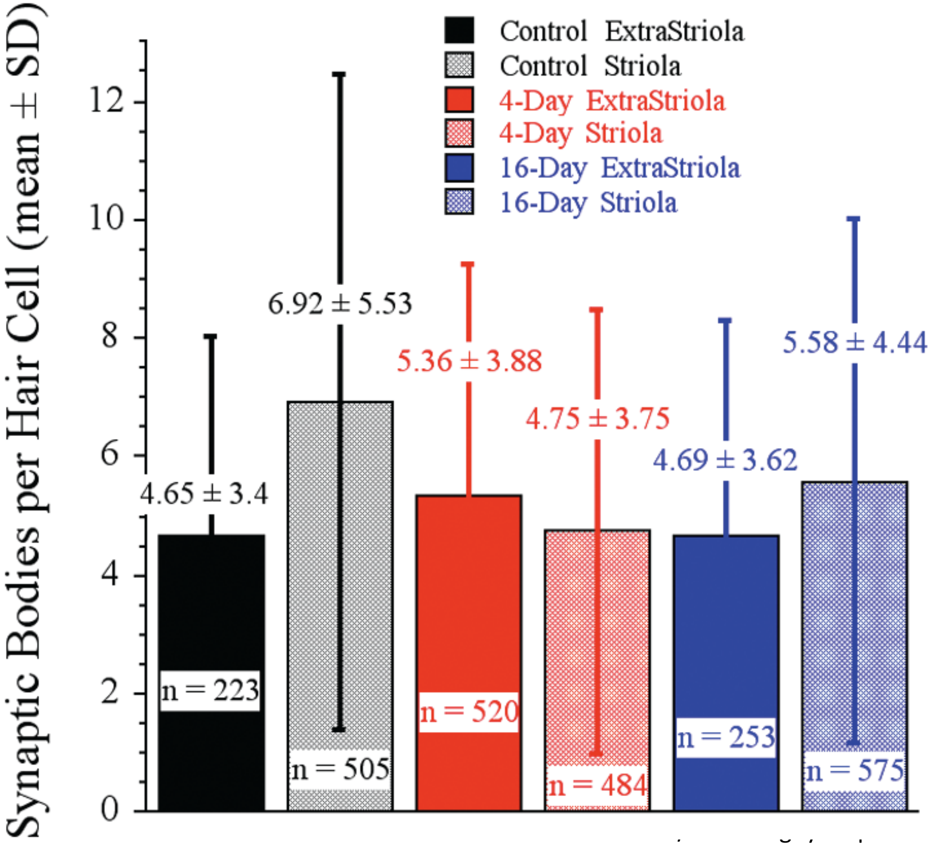
Afferent Smax as a function of days of exposure to 2.24g and readaptation after return to normal 1g. **(A)** Same data as shown previous slide. **(B)** Histogram plots afferent Smax as a function of number of days (indicated by number inside of each column) in normal 1g after 4- and 16-day exposures to centrifugation to the return to baseline levels. ***Initial hypersensitivity*** recorded immediately after 4-day exposures (solid red unlabeled column) required >4 days to recover to control levels. ***Later hyposensitivity*** observed after 16-day exposures (solid blue unlabeled column) required at least 2 days to recover. In each panel error bars are SD, the left column labeled **C** is the control response values, and asterisks designate the level of significance of $p < 0.05$ (*), $p < 0.005$ (**), and $p < 0.0001$ (***) to control measures.

Do the number of synaptic ribbons in hair cells determine afferent sensitivity following hyper-g?



We selected 2 animals in control, 4D, and 16D groups and counted synaptic bodies in 2 locations of the macula using serial reconstructions from transmission electron micrographs. Note the physiological separation of the controls. NO significant differences were found.





Summary: Influence of Altered Gravity on the Peripheral Vestibular System in

- Otolith afferents recorded after return to Earth exhibited a profound *hypersensitivity* to translational accelerations, and recovery to normal level was within 1-2 days. It is reasonable to suggest that astronaut behavior following a short-term exposure to µg reflects the physiological changes occurring in the otolith structures.

- A biphasic pattern in response to hyper-g is observed: an *initial hypersensitivity* (3- to 4-day), similar to that observed in otolith afferents upon return to 1g, followed by a transition through normal sensitivity to a significant *hyposensitivity* at 16- to 32-day exposure. Return to control values following 4- and 16-day exposure to 2.24 g is ~2-6 days. The initial hypersensitivity might reflect the more common transient symptoms experienced by astronauts in the early stages of the mission.

Summary: Influence of Altered Gravity on the Peripheral Vestibular System in Vertebrates

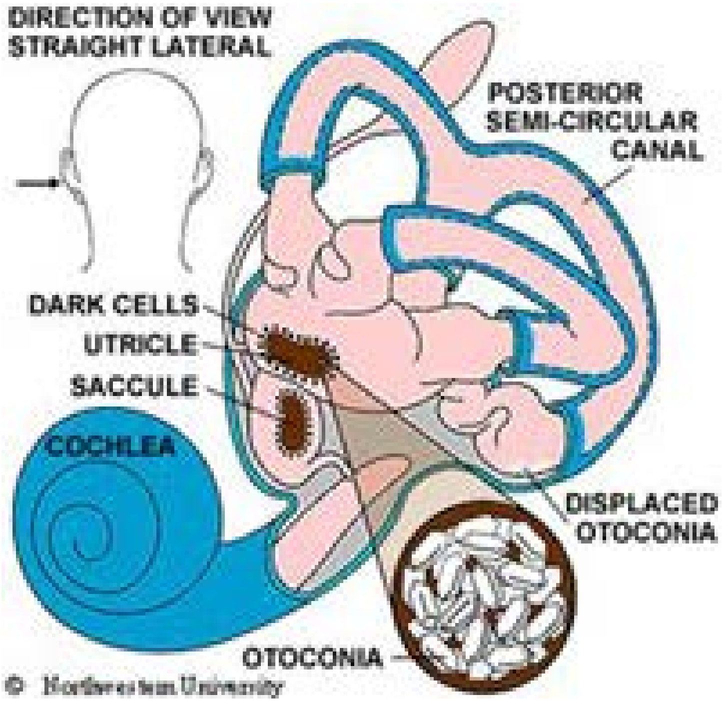
- *Synaptic Plasticity.* Of importance, no significant changes in hair cell synaptic ribbon counts were found in animals where highly significant differences were observed in afferent sensitivity.

- *Animal behavior* within the first day post-flight and within the first days of hyper-G was noted: a hyper-excitable state and a reluctance to move. This initial behavior to *altered gravity* (AG) likely reflects the hypersensitivity of otolith nerve afferents. *It is possible* that AG *initiates first* a large gain increase in response to a challenge, any AG challenge. Using animal behavior as an indicator of the underlying otolith afferent physiology, intermittent or partial AG exposure on ISS might be applied to query neurovestibular changes and validated parameters as potential countermeasure protocols.

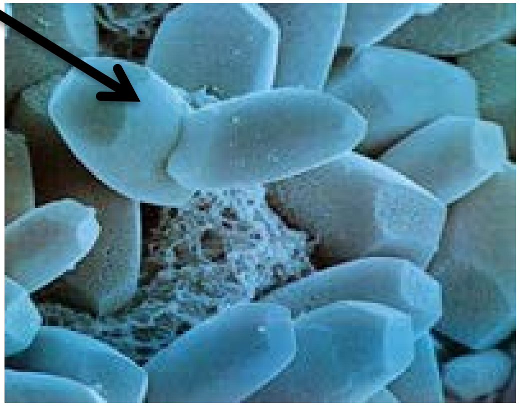
Boyle R, Popova Y, Varelas J. (2018) Influence of magnitude and duration of altered gravity and readaptation to 1g on the structure and function of the utricle in toadfish, *Opsanus tau*. Front. Physiol. 9:1469. doi: 10.3389/fphys.2018.01469.

CaCO$_3$ is used by both invertebrates and vertebrates to help transform acceleration into a

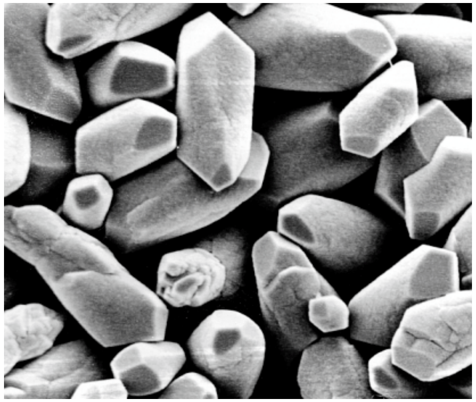
Mass of otoconia **increases the inertia** of the system



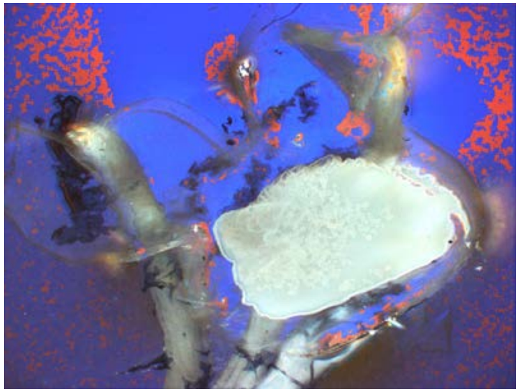
Human Otoconia



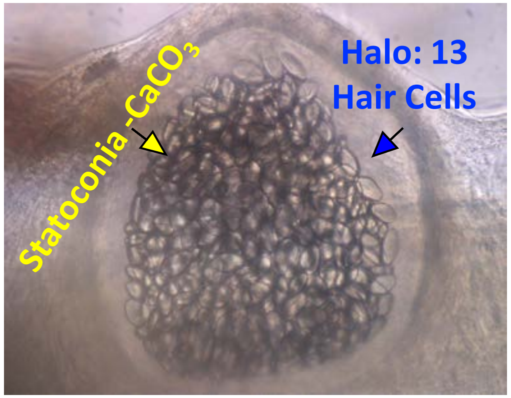
Rodent Otoconia



Toadfish Otolith



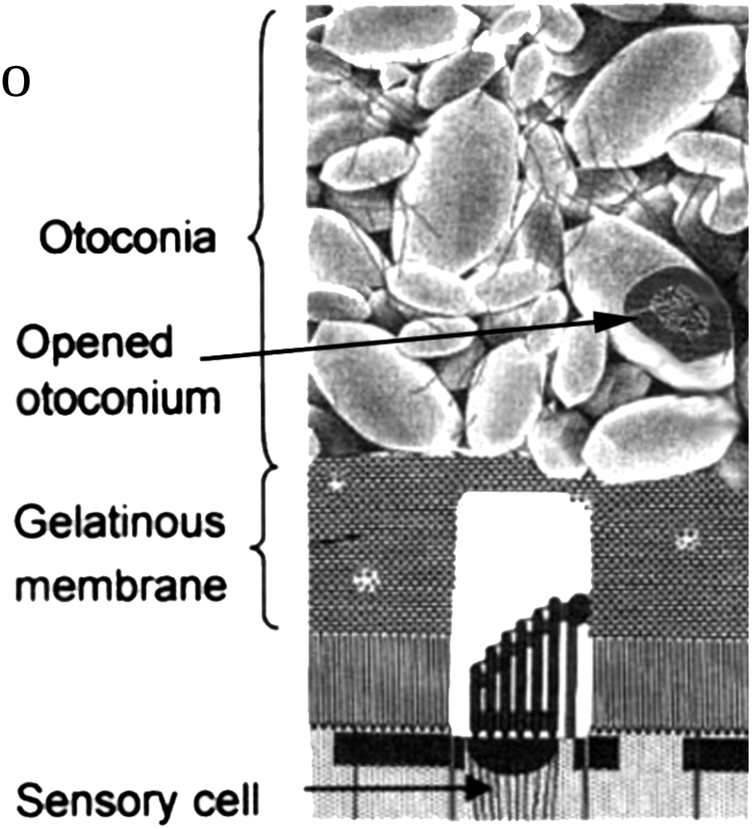
Snail Statoconia



Mouse Inner Ear Otoconia

Key Points

- ***Weight-lending*** Otoconia provide **mechanical loading** of the vestibular hair cells' bundles
- Their high density ***increases*** **sensitivity** to linear acceleration and change in orientation re: gravity
- **Inorganic shell** is $CaCO_3$; **inner core** is an **organic matrix** of protein
- Because of low endolymphatic levels of *Ca*$^{2+}$ *and* CO_3^{2-} *ions, efficient concentrating mechanisms* mediated predominantly by glycoproteins are required for crystal nucleation and growth.



From Lins et al. *J Struct Biol* 131:67–78, 2000.

Key Points

- **Otoconia function is not fixed**. The shell can turn over. They slowly and progressively degenerate in aging, resulting in loss of balance and falls in elderly patients.
- Clinical syndromes of *canalithiasis* and cupulolithiasis, the most common single cause of vertigo, are biomechanical in origin and occur when otoconia fragments are displaced from their normal location.
- Despite the significant morbidity, little is known about the structural and molecular processes involved in otoconial development, maintenance, and pathology. This is particular true for the influence of space flight on otoconia structure.

Study Overview

Question: Is the structure of otoconia remodeled by the intensity and duration of gravity loading?

Samples:

1) mice flown on 90-day Mouse Drawer System mission to ISS

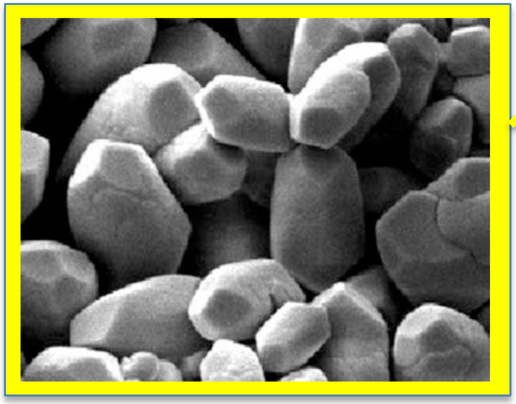
2) equivalent MDS mouse models exposed to 1.4G (=√2G) centrifugation and hindlimb unloading for 90 days; and
3) mice flown on STS-133 and -135 missions of ~13-day duration

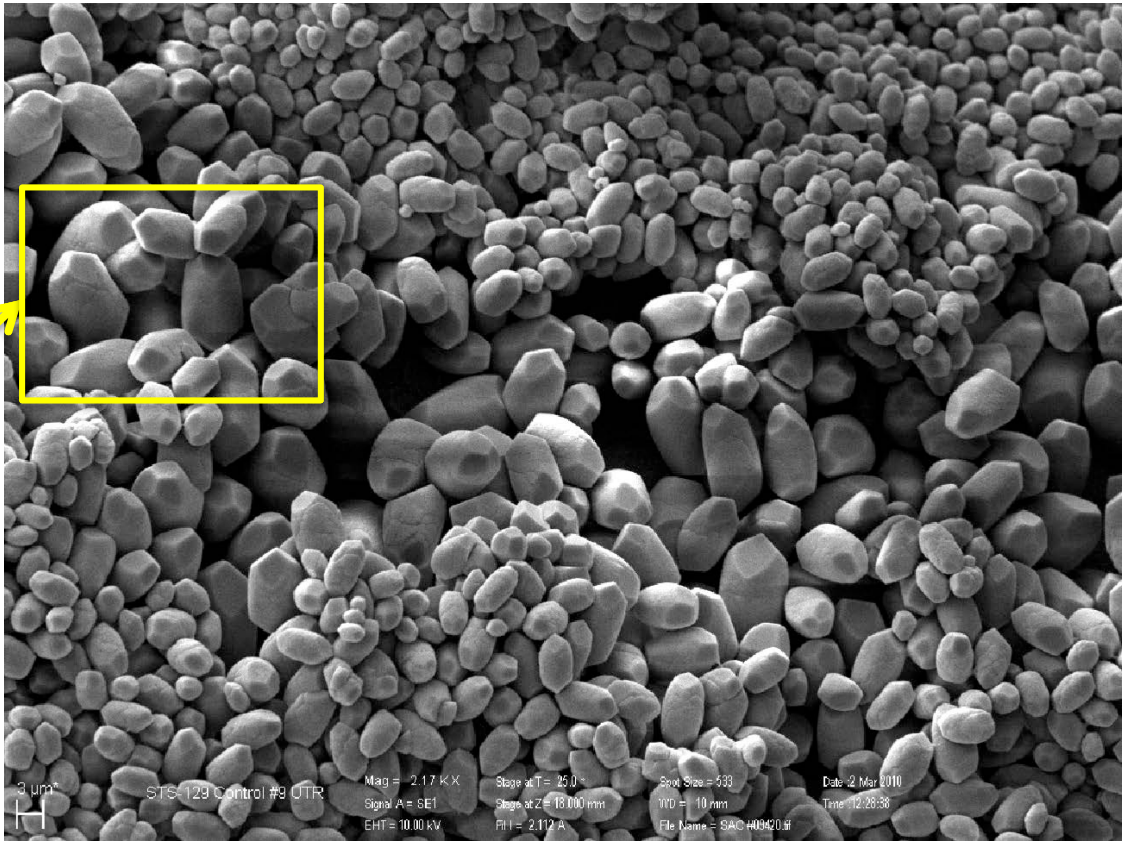
Answer: Scanning electron microscopy with combined Focus Ion Beam milling evidence *suggests* -- Yes (conditional)

Mechanism: A constructive disposition in low G and ablative removal in high G of $CaCO_3$, possibly under pH control of the endolymph. This mechanism needs experimental validation.

Otolith otoconia at 1G normal in Mice

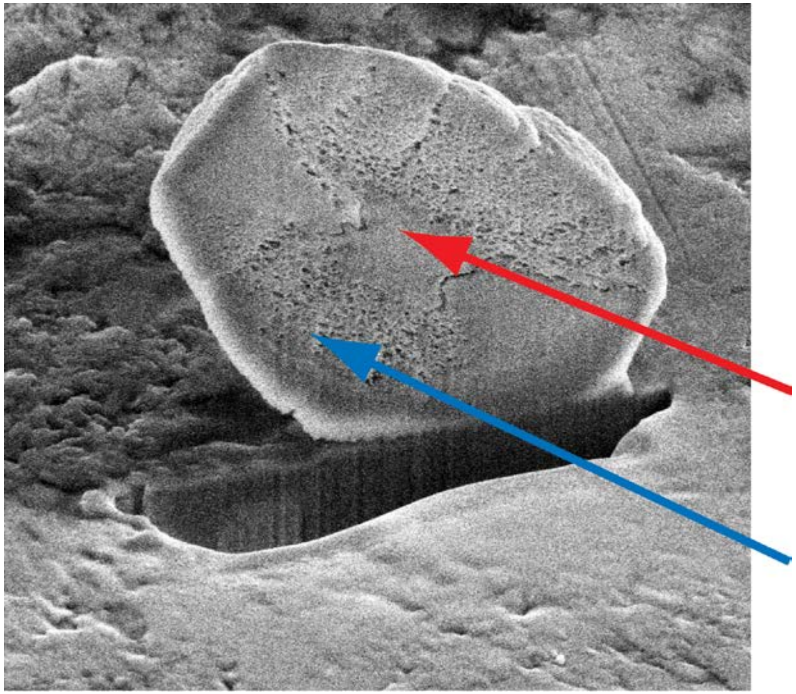
MDS Otoconia-Control



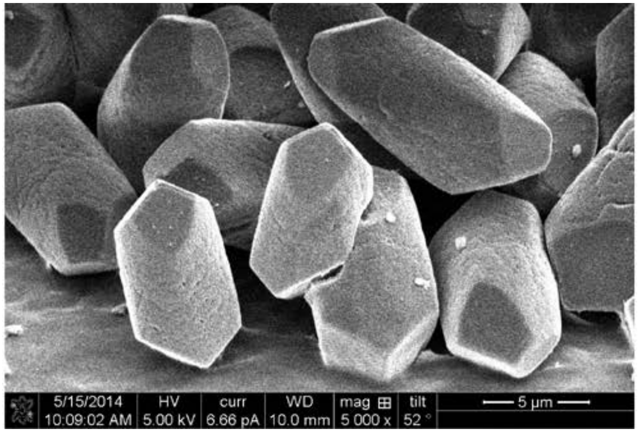


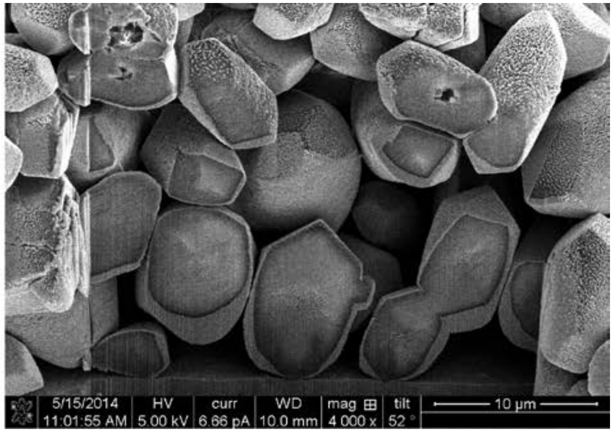
Utricular mass from flight control shows normal hexagonal symmetry. Other controls showed normal morphology of both the utricle and saccule, indicating that age and housing conditions cannot explain the difference in flight and control mice.

Otoconia at 1G normal in control Mice



Inner core and outer shell transition boundary. Focused Ion Beam (FIB) milling reveals inner core and outer shell of a single grain.

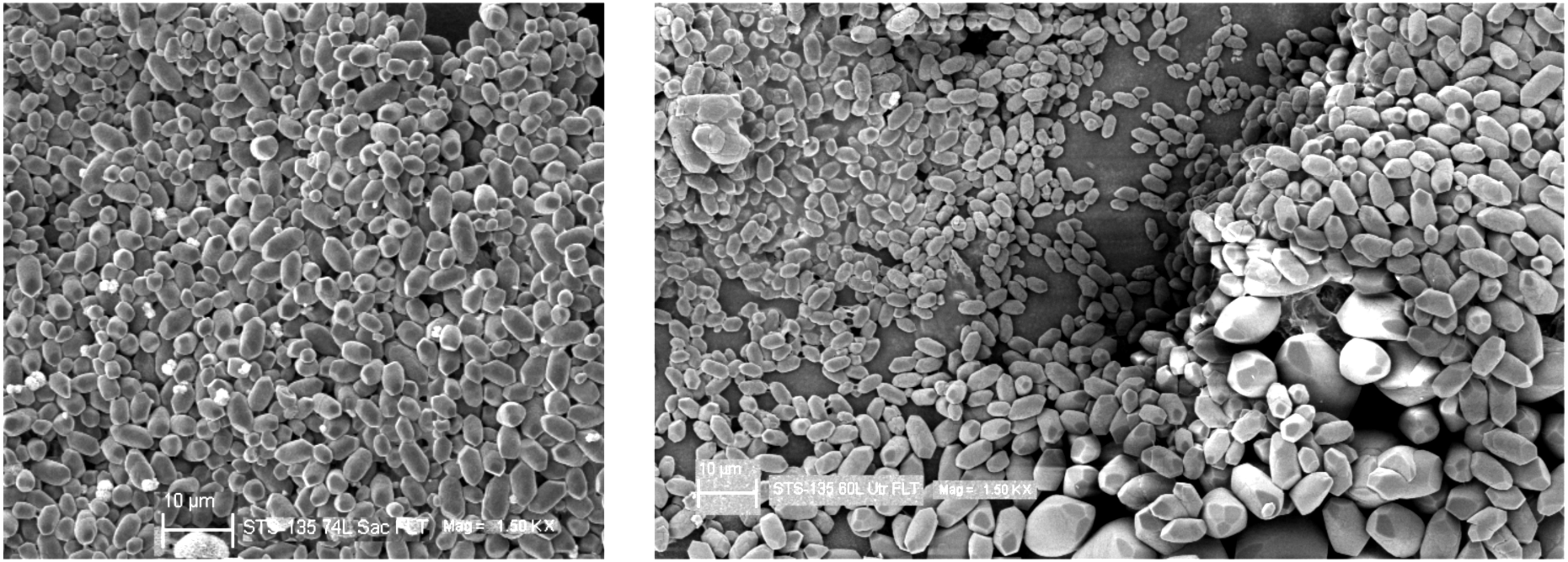




Pre-milling (left) and FIB-milled (right) images show normal consistency of control otoconia.

Influence of Reduced Gravity of Short Duration on Otoconia in Mice

STS-135 Otoconia-Flight

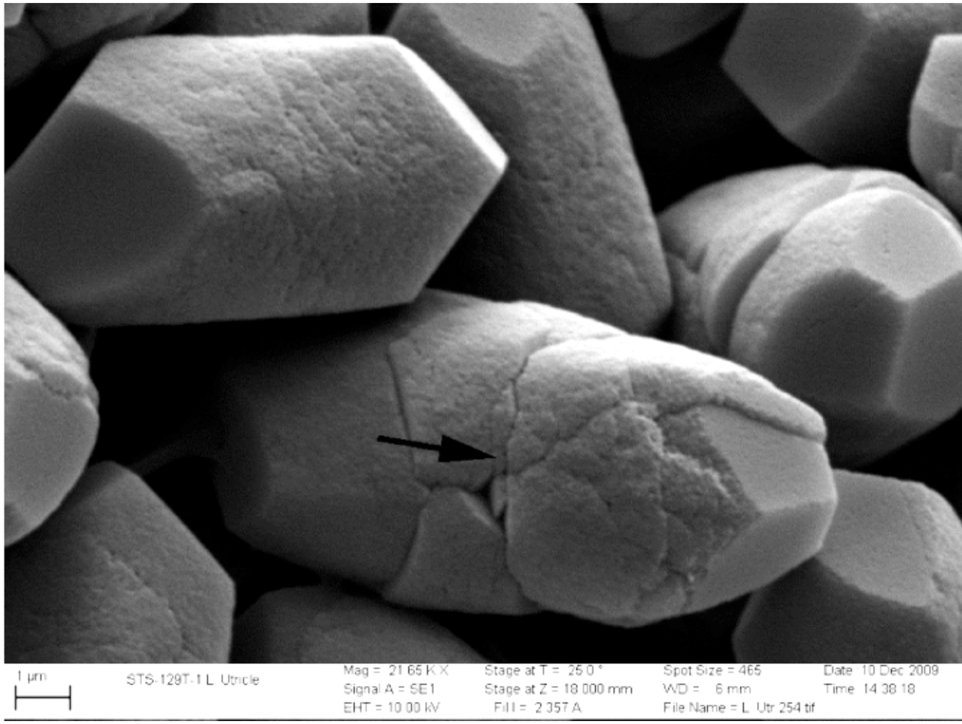


Saccular (left panel) and utricular (right panel) from 2 separate mice flown on the 13-day STS-135 mission. Otoconia appear normal in the samples [1.5kX magn.]

Influence of Reduced Gravity of long Duration on Otoconia in Mice

MDS Otoconia-Flight

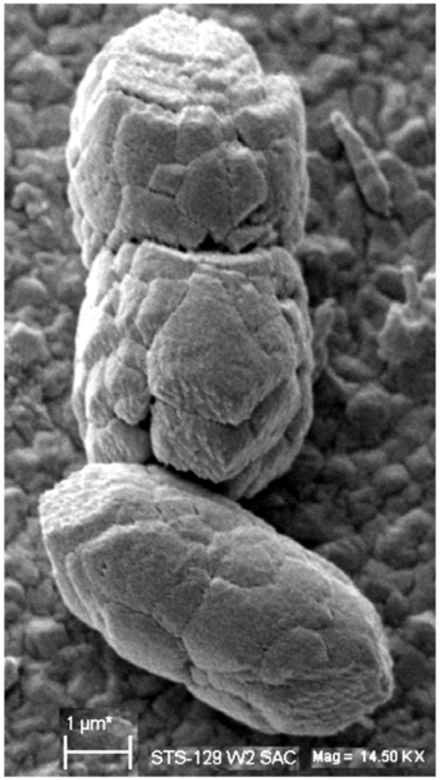
SEM image of utricular otoconia from 90-day MDS flight Mouse. Arrows indicate regions of assumed restructuring of the individual particle. [8kX magn.]

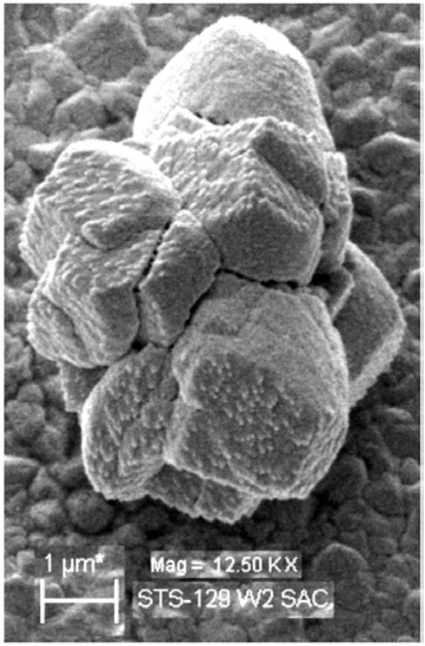


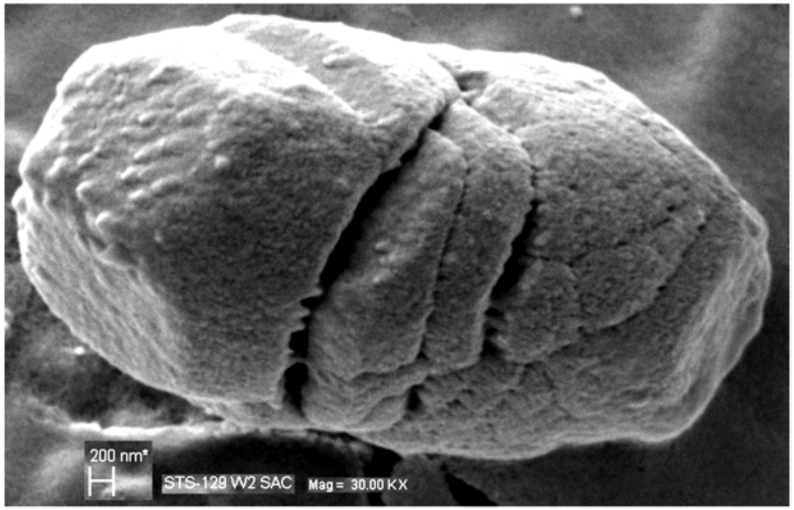
Higher magnification of individual particles reveal possible areas of calcium deposition (see arrow). [21.65 kX magn.]

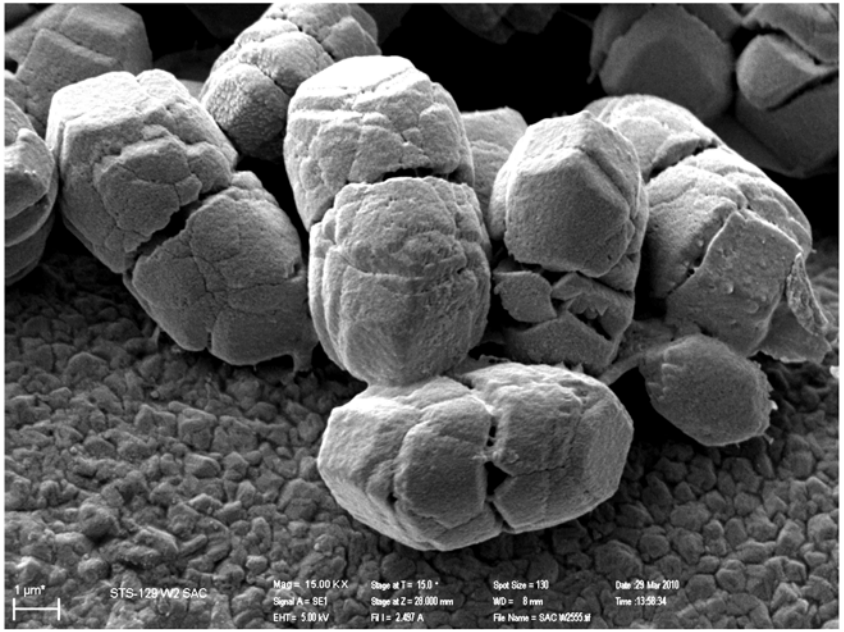
Influence of Reduced Gravity of long Duration on Otoconia in Mice

MDS Otoconia-Flight





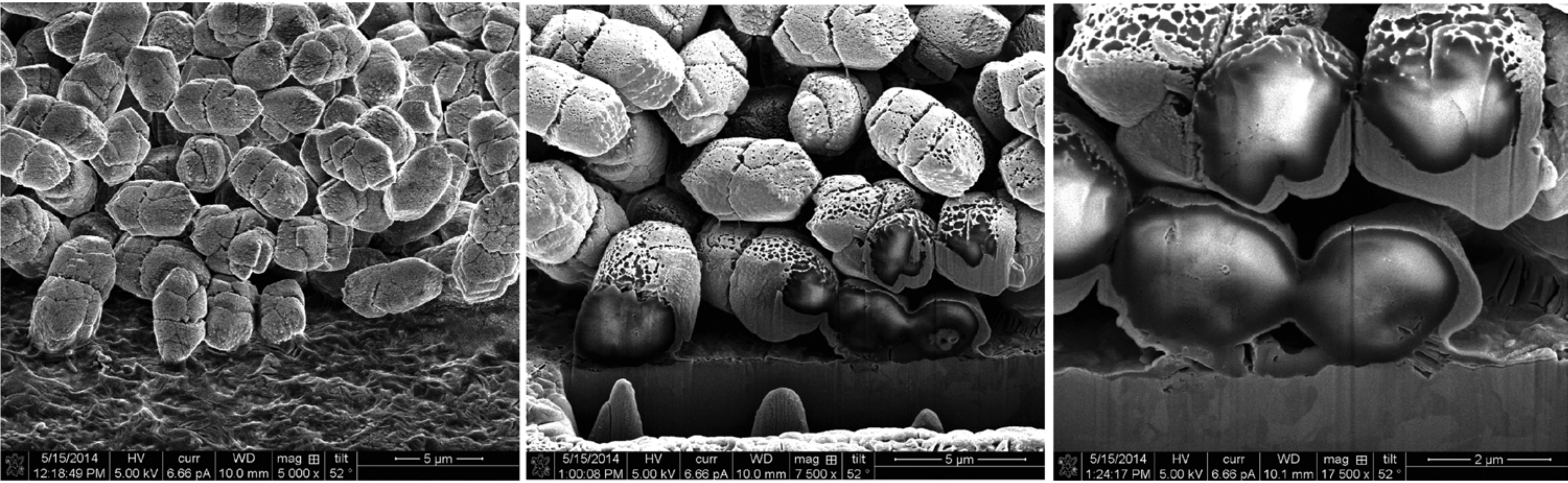




Saccular otoconia from a flight mouse show comparable configuration changes as seen in the utricle

Influence of Reduced Gravity of long Duration on Otoconia in Mice

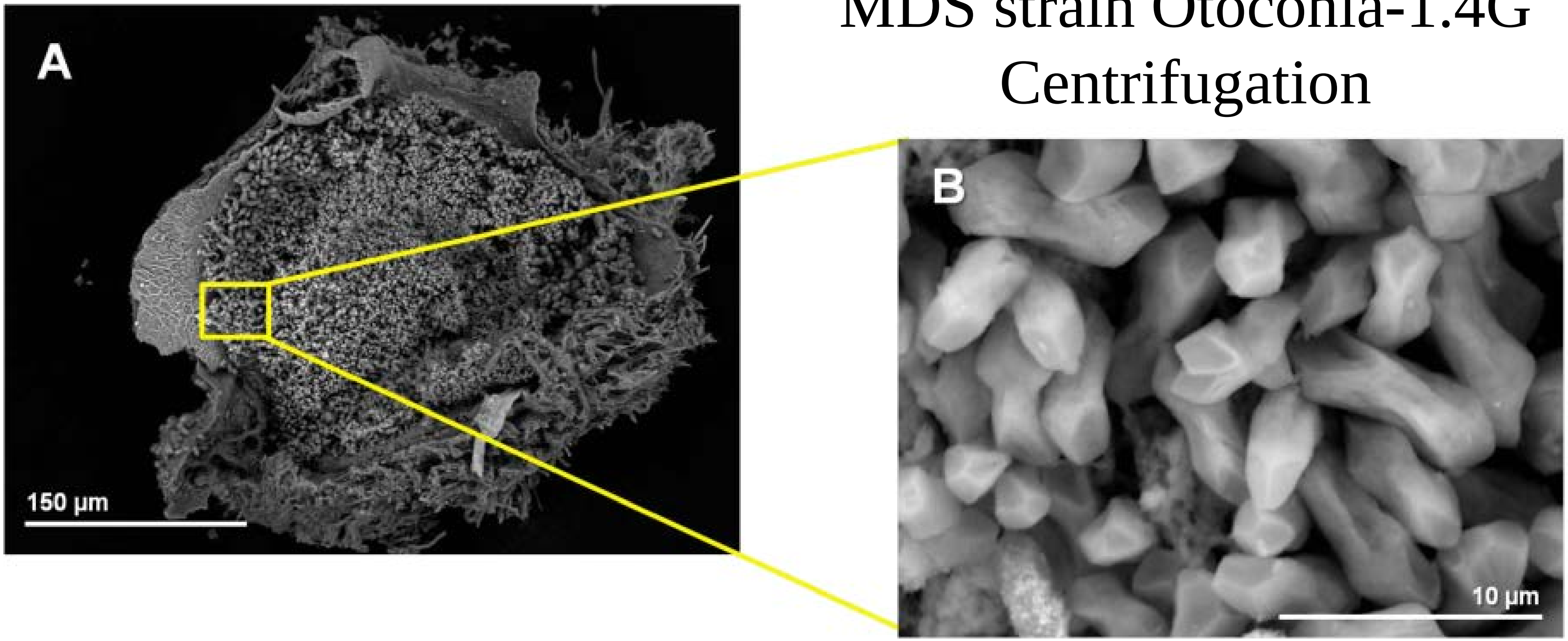
MDS Otoconia-Flight



Another example of the consequence of µG exposure for 90 days on the structural integrity of otolith otoconia. SEM images taken before (left) and after FIB milling (middle, right) show the thickening on the outer shell and a more solid, dense inner core, suggesting a constructive process of disposition after prolonged weightlessness.

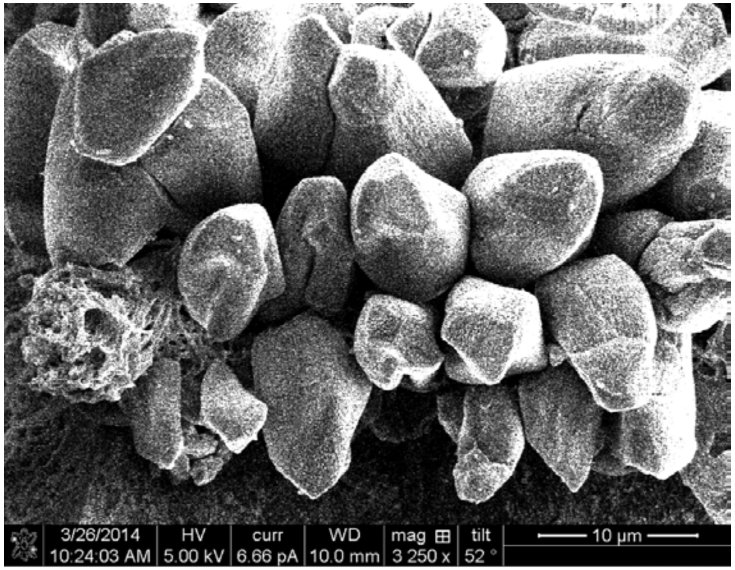
Influence of Increased Gravity of long Duration on Otoconia in Mice

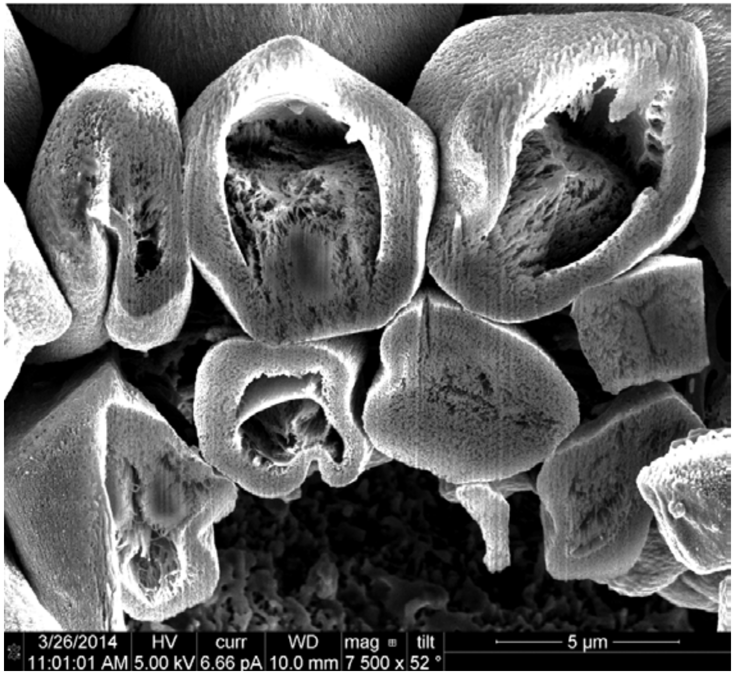
MDS strain Otoconia-1.4G Centrifugation



SEM analysis of mouse 1.41G-centrifuged otoconia. (A) Dissected utricle shows otoconia. (B) Enlarged view of otoconia from indicated area. Note the "dumbbell"-like appearance of otoconia. Samples were fixed, dehydrated, critical point dried, mounted and examined on a Hitachi TM-1000 Tabletop SEM.

Influence of Increased Gravity of long Duration on Otoconia in Mice





MDS strain Otoconia-1.41g Centrifugation

Another example of the consequence of exposure to an increased gravity environment on the structural integrity of otolith otoconia. Pre-milling image (top) and FIB-milled otoconia (bottom) show **cavitation of inner core** and *thinning of outer shell*. Although cavities are often seen in otoconia, they are exceptional in extent in the 90-day 1.41g mice.

Summary

Importance of gravity sensation on life is mostly studied in the framework of the consequences of its loss on motor and equilibrium function seen in patients. Now that humans have ventured into space above the influence of Earth's mass, we now know that animals, including humans, adaptively respond (often similarly) to novel gravity states.

Images from 90-day flight mice indicate a restructuring of the otoconia, suggesting disposition to the outer shell. Images from their counterparts exposed to 90-day centrifugation at 1.4g indicate the converse - a thinning of the otoconia outer shell and the inner core is more cavitated. For shorter duration µg exposures and hindlimb unloading otoconia appear normal.

Despite the permanence of Earth's gravity (1g) in evolution, our research indicates that the animal senses an exposure to a novel, non-1g, environment and adaptive neural mechanisms are initiated to restore normalcy- in the short term compensation is likely confined to peripheral sensory receptors of the inner ear, the brain or both. If the gravity level persists without intervention for a prolonged duration, structural changes might result.

Site(s) of Plasticity driving Neurovestibular Adaptation?

Otoconia Layer

Anken, Wiederhold: change in mass
Boyle et al: Otoconia changes in long duration exposure
Takumi et al: S100a8,9 (calgranulin A,B) and
Oc90 (Otoconin90) gene expressions

Gel Layer ?

Stereocila: Ion Channels (K^+, Ca^{2+})

Good candidates, only indirect evidence

Stereocila – Hair Cell Linkage

Possible but no direct evidence

Hair Cell or Hair Cell-Afferent Synapse

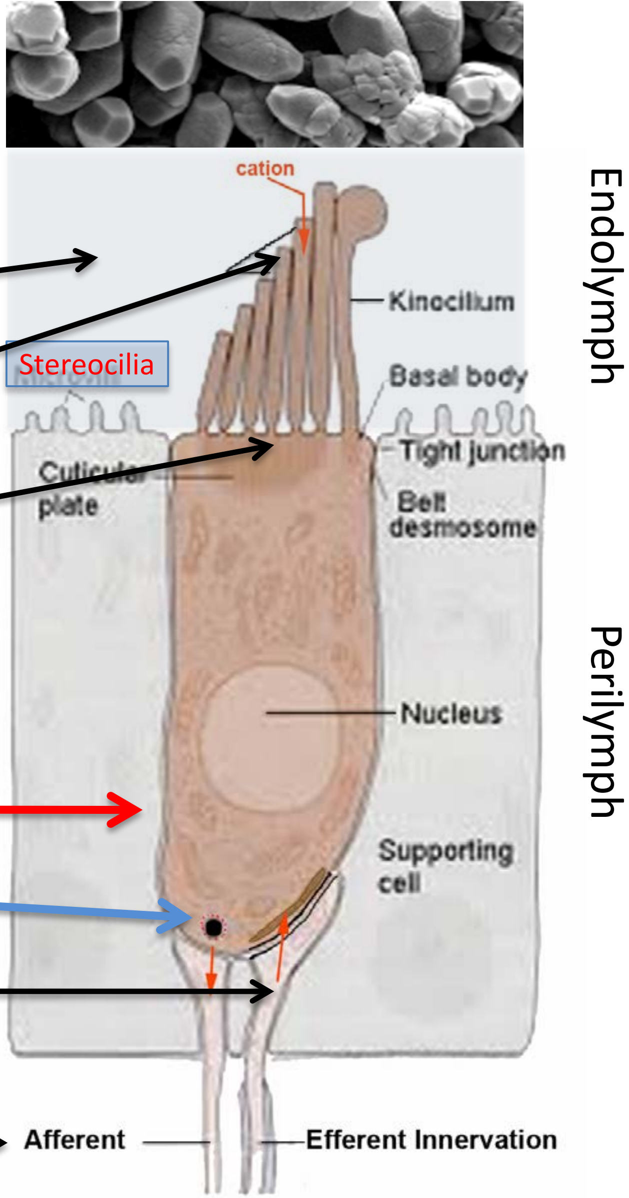
Balaban et al: change in invertebrate hair cell sensitivity
Ross: change in synaptic density
Boyle et al. (2001): change in afferent sensitivity
Boyle et al. (2018): no correlation with sensitivity changes

Efferent Synapse

Possible candidate, but likely not related to constant change

Afferent Morphology

Good candidates, but no direct evidence



Short vs Long Duration of Exposure – is there a Real Concern?

Short-Duration Altered Gravity Exposure

Neural Plasticity:

√Afferent Hypersensitivity (µG: Bracchi et al. 1975)
√ Hair Cell Synaptic Structure (µG: Ross 1993)
√ Afferent Hypersensitivity (µG: Boyle et al. 2001)
√ Afferent Plasticity (HG: Boyle et al. 2018)
ø Hair Cell Synaptic Plasticity (HG: Boyle et al. 2018)

Structural Otoconia Plasticity:

√ ~Evidence in fish/mollusks (Anken, Wiederhold…)
ø SEM evidence in mice from STS-133 or 135 (Boyle)

Long-Duration Altered Gravity Exposure

Neural Plasticity:

√ Afferent Sensitivity (HG:Boyle et al. 2018)

Structural Plasticity:

√ Otoconia Changes (MDS mission, Boyle)
√ Otoconin-90 changes (otoconia matrix protein – Takumi et al)
√ Constructive/Destruction Process: Supported by centrifugation data

Thank you

- Special Appreciation to the NASA’s Human Health and Countermeasures (HHC), Human Research Program (HRP) and Space Biology Program; the Mouse Drawer System (MDS) of the Italian Space Agency (ASI); and our friend Yoshi Ohira at University of Osaka, Japan.

Site(s) of Plasticity driving Neurovestibular Adaptation?

Otoconia Layer

Anken, Wiederhold: change in mass

Boyle et al: Otoconia changes in long duration exposure

Takumi et al: S100a8,9 (calgranulin A,B) and Oc90 (Otoconin90) gene expressions

Gel Layer ?

Rapid or Short-term Action

Stereocila: Ion Channels (K^+, Ca^{2+})

Good candidates, but no direct evidence

Stereocila – Hair Cell Linkage

Possible but no direct evidence

Hair Cell or Hair Cell-Afferent Synapse

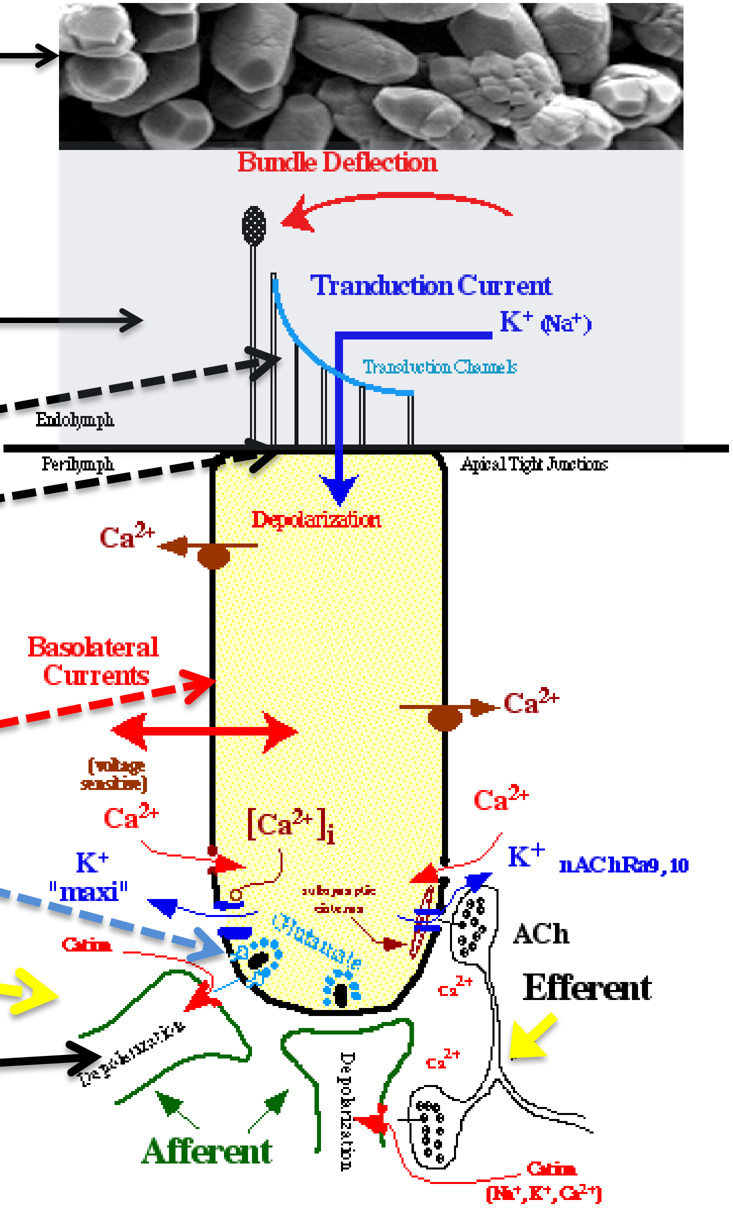
Balaban et al: change in invertebrate hair cell sensitivity

Ross: change in synaptic density

Boyle et al.: change in afferent sensitivity

Efferent Synapse

Good candidate, but no direct evidence



Afferent Morphology

Good candidates, but no direct evidence