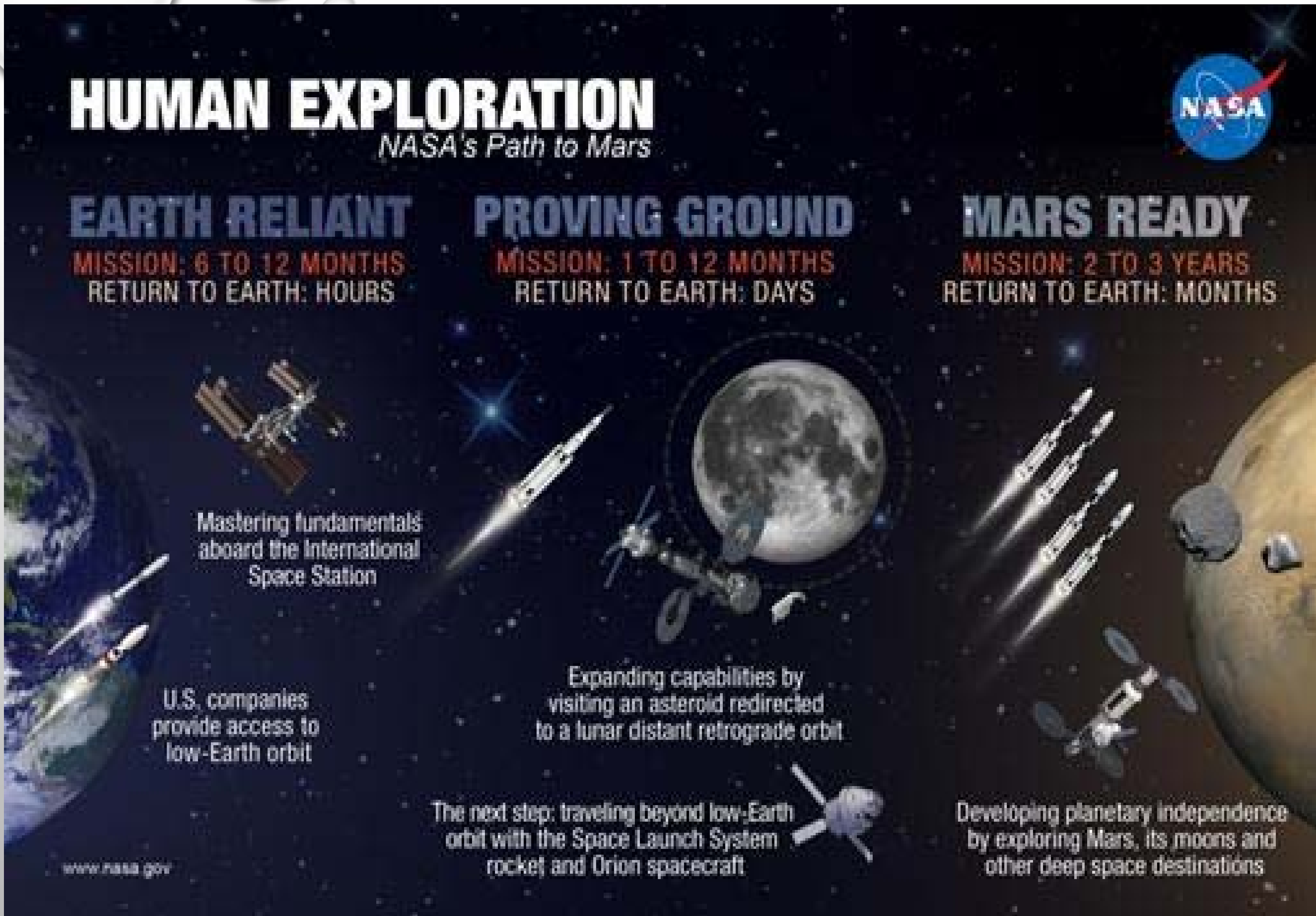
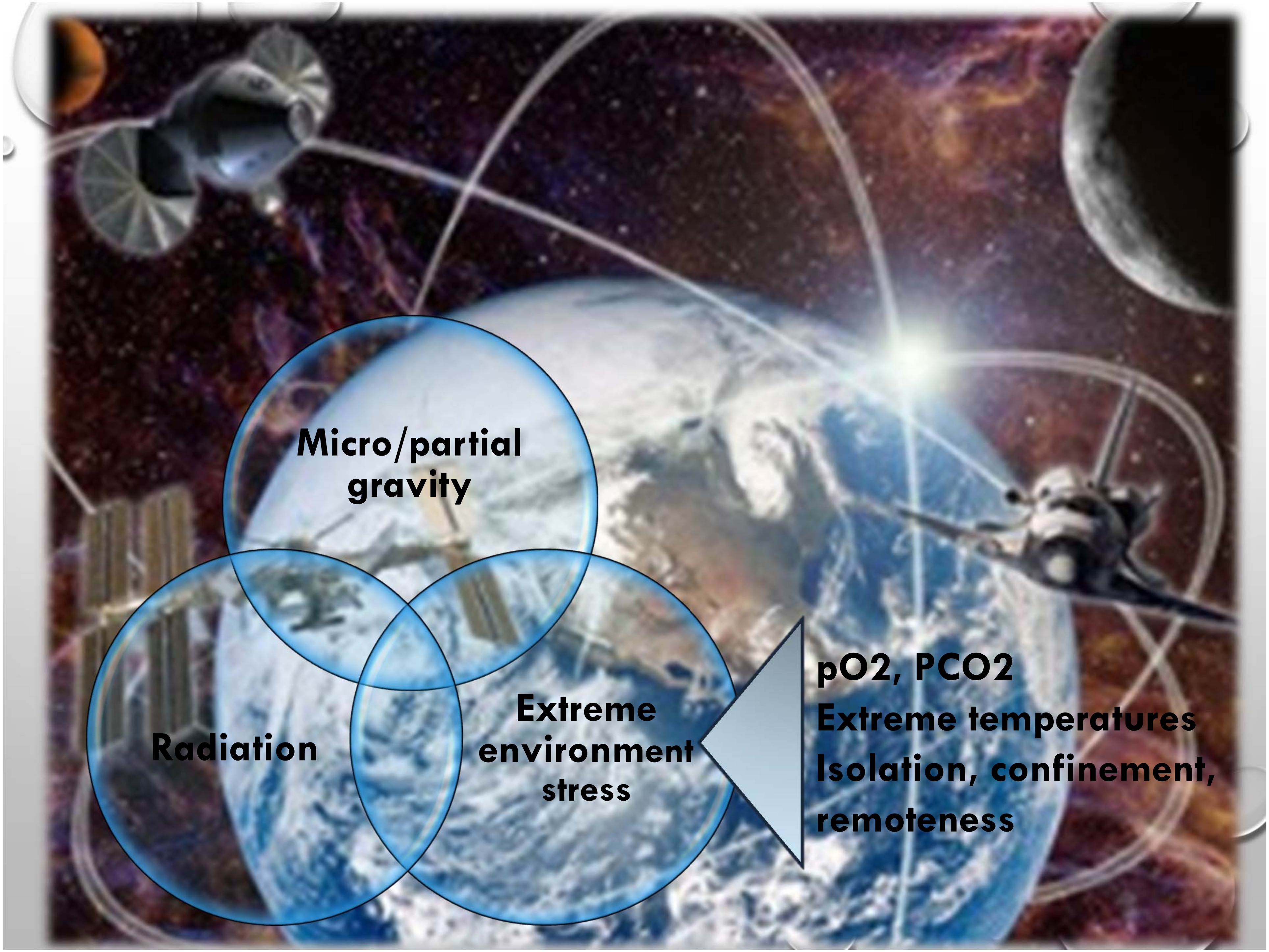
Brain and Ocular Changes in Space

Susana B. Zanello, Ph.D., Universities Space Research Association (USRA)
NASA Johnson Space Center


HUMAN EXPLORATION
NASA's Path to Mars
NASA
EARTH RELIANT
MISSION: 6 TO 12 MONTHS
RETURN TO EARTH: HOURS
Mastering fundamentals aboard the International Space Station
U.S. companies provide access to low-Earth orbit
PROVING GROUND
MISSION: 1 TO 12 MONTHS
RETURN TO EARTH: DAYS
Expanding capabilities by visiting an asteroid redirected to a lunar distant retrograde orbit
The next step: traveling beyond low-Earth orbit with the Space Launch System rocket and Orion spacecraft
MARS READY
MISSION: 2 TO 3 YEARS
RETURN TO EARTH: MONTHS
Developing planetary independence by exploring Mars, its moons and other deep space destinations
www.nasa.gov

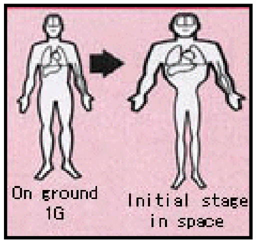

Micro/partial gravity
Radiation
Extreme environment stress
pO2, PCO2
Extreme temperatures
Isolation, confinement, remoteness

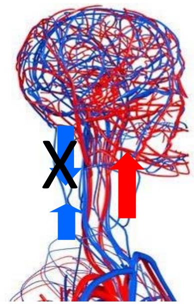
HUMAN RESEARCH ROADMAP

- HRP USES AN INTEGRATED RESEARCH PLAN TO IDENTIFY THE APPROACH AND RESEARCH ACTIVITIES PLANNED TO ADDRESS THESE RISKS
- [HTTP://HUMANRESEARCHROADMAP.NASA.GOV/](http://humanresearchroadmap.nasa.gov/)

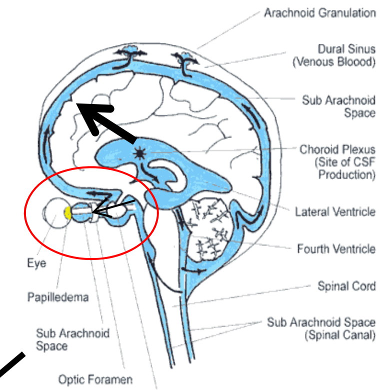
VIIP PROPOSED PATHOPHYSIOLOGY

1. Weightlessness-induced headward fluid shift



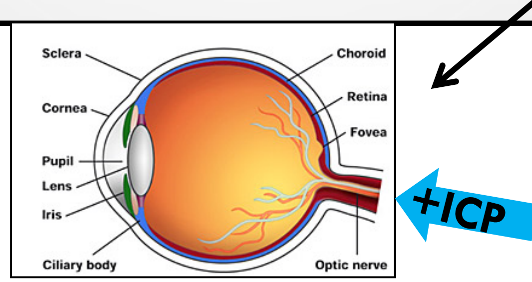


2. Fluid shift increases intracranial pressure (ICP)

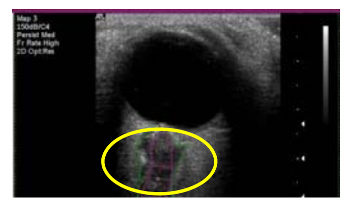


3. Elevated ICP & fluid shift transmitted to the eye

Hyperopic Shifts
Up to +1.75 diopters

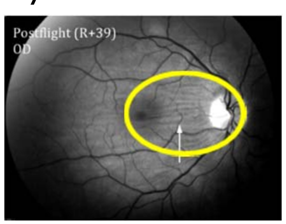


Increased Optic Nerve Sheath Diameter



"Cotton wool" Spots - Altered blood flow

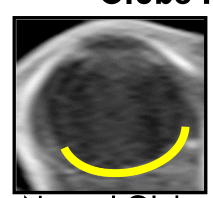
Choroidal Folds
Ridges in back of eye

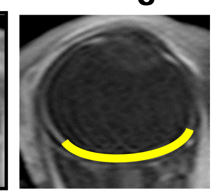


Optic Disc Edema
(Swelling)

Scotoma
Abnormal visual field

Globe Flattening


Normal Globe


Flat Globe

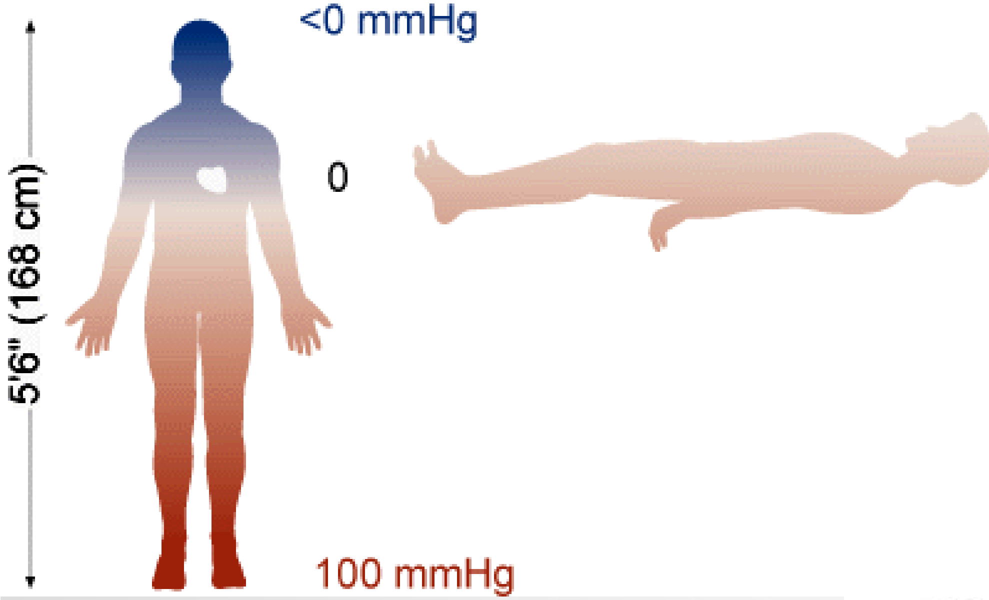
Courtesy Christian Otto, MD, HRP VIIP Project Scientist, July. 2014

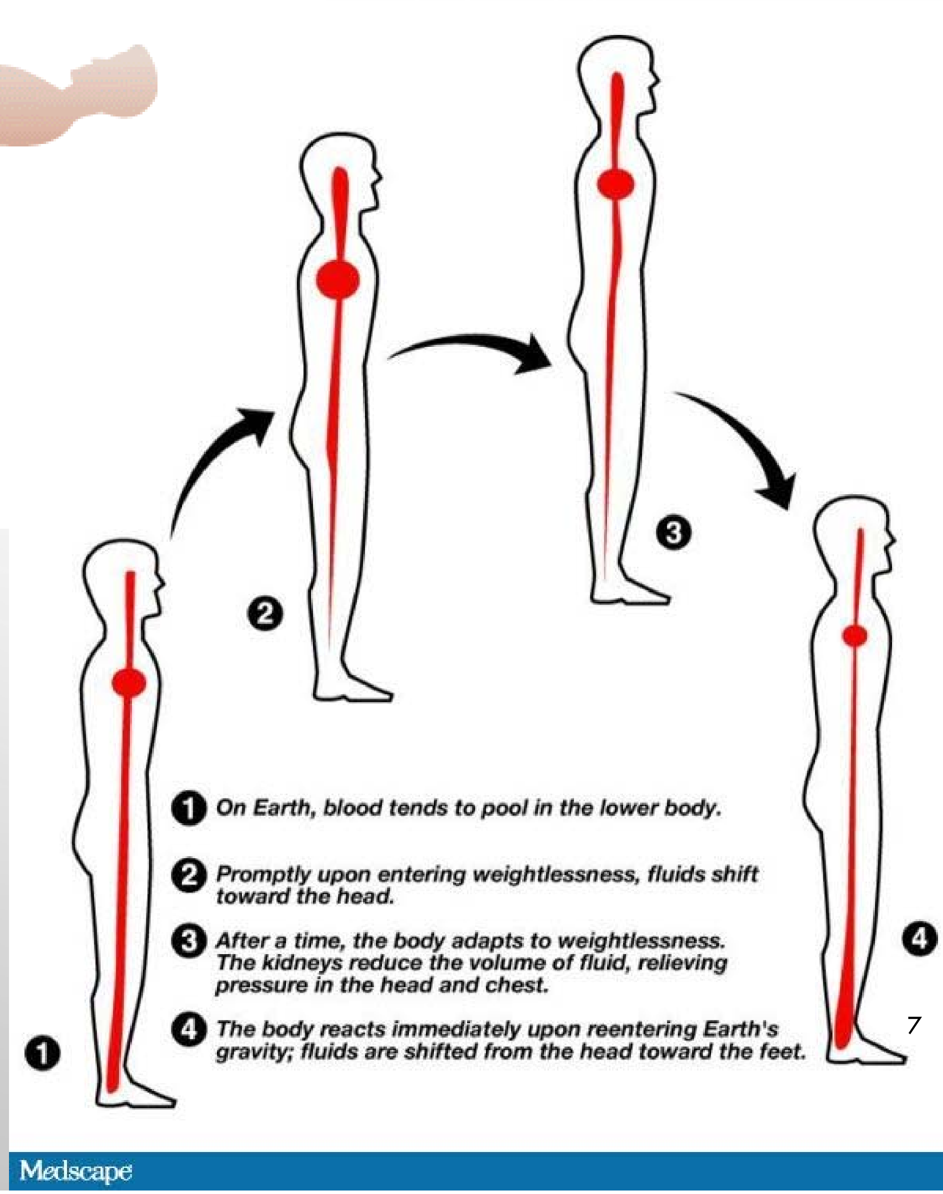
RISK OF SPACEFLIGHT-INDUCED INTRACRANIAL HYPERTENSION/VISUAL ALTERATIONS

Risk Rating	
ISS-12	Uncontrolled
Lunar	Insufficient Data
Deep Space Journey	Uncontrolled
Planetary	Uncontrolled

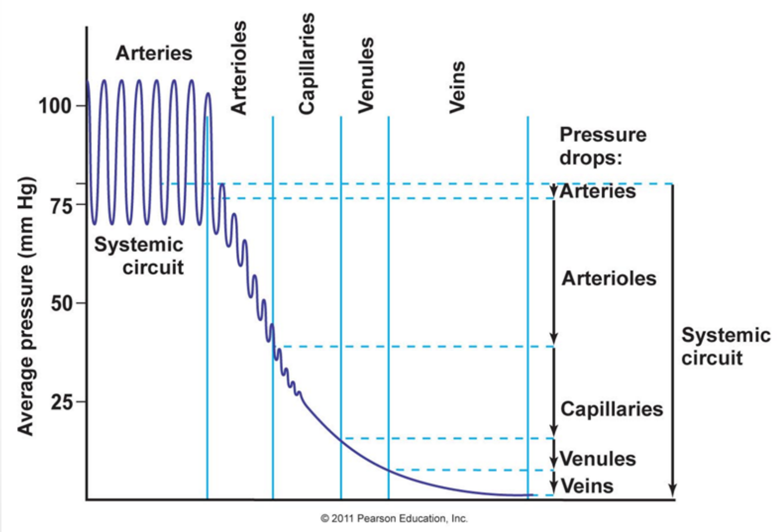
- *VIIP1: We do not know the etiological mechanisms and contributing risk factors for ocular structural and functional changes seen in-flight and postflight.*
- *VIIP3: We need a set of validated and minimally obtrusive diagnostic tools to measure and monitor changes in intracranial pressure, ocular structure, and ocular function.*
- *VIIP12: We do not know whether ground-based analogs and/or models can simulate the spaceflight-associated VIIP syndrome.*
- *VIIP13: We need to identify preventive and treatment countermeasures (CMs) to mitigate changes in ocular structure and function and intracranial pressure during spaceflight.*

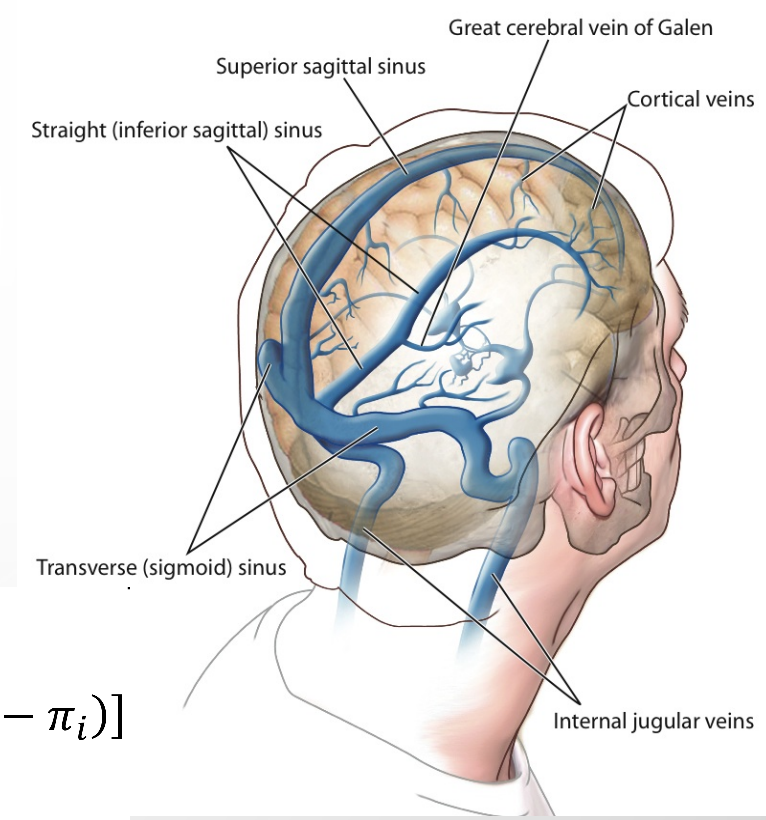
CEPHALAD FLUID SHIFT

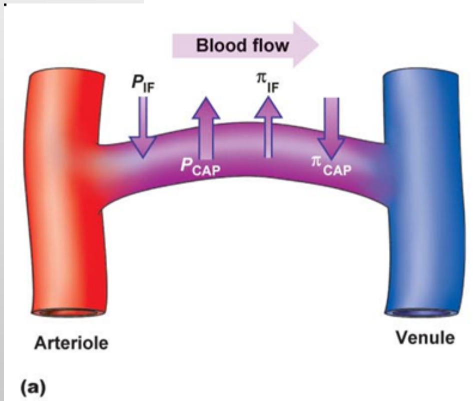




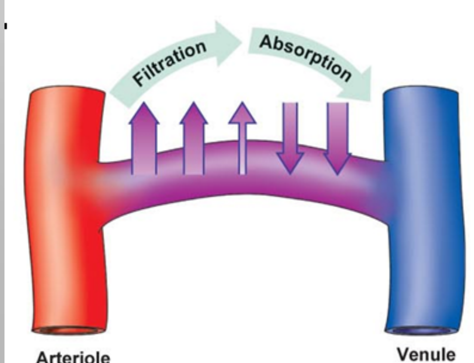
www.nasa.gov/exploration/humanresearch







$$J_v = K_f[(P_c - P_i) - \sigma(\pi_c - \pi_i)]$$



Fluid movement into or out of the capillaries is dependent on the hydraulic pressure drop ($P_c - P_i$) minus the colloid osmotic pressure drop ($\pi_c - \pi_i$). The filtration coefficient (K_f) takes into account the permeability of the capillary membranes to water which is dependent on surface area and hydraulic conductance. The reflection coefficient (σ) is used to correct for the fact that not all plasma proteins are effective in retaining water, and is different in various vascular beds.

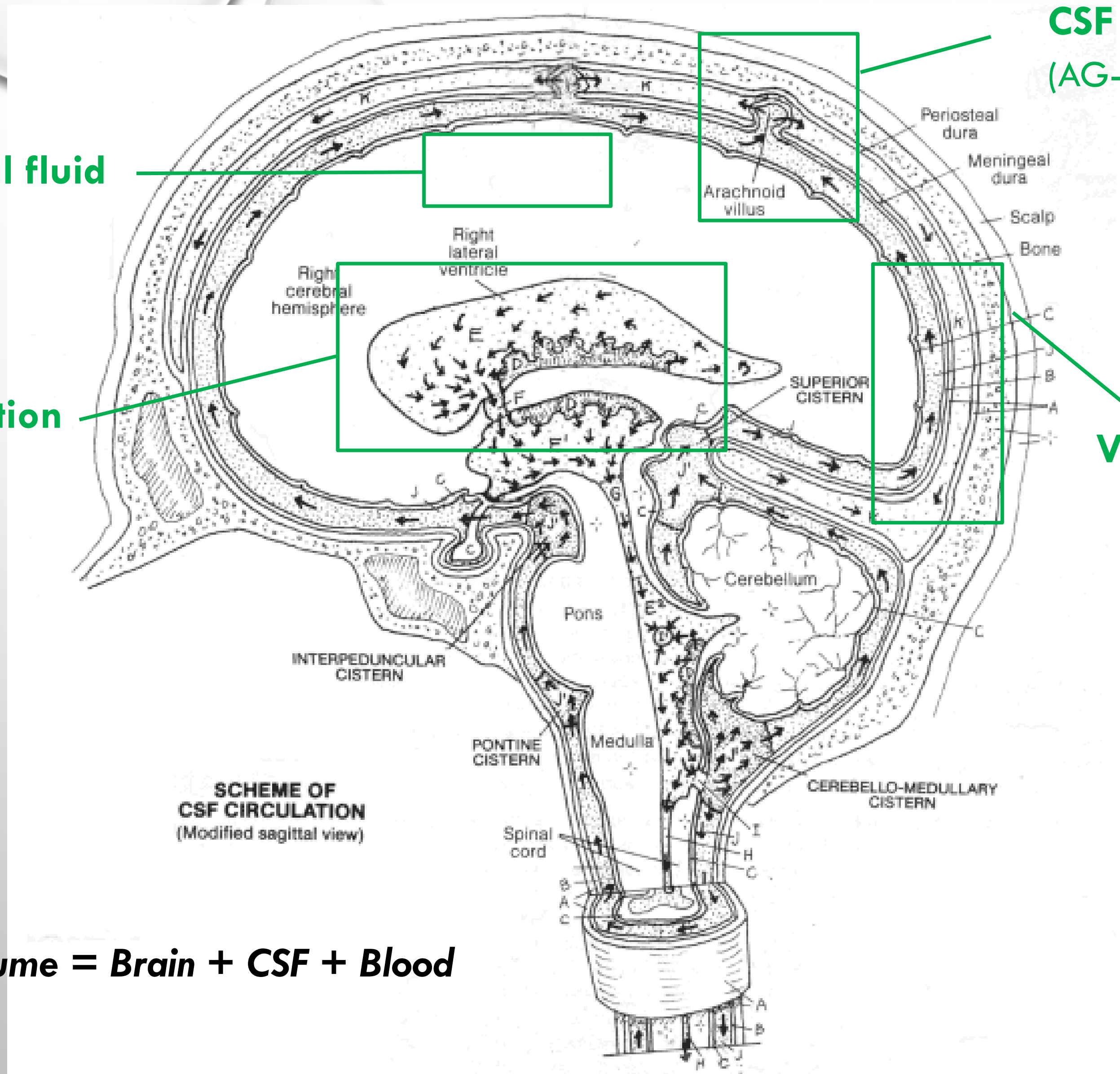
CSF Resorption

(AG-Venous/Lymphatic)

Interstitial fluid

CSF Production

Venous Congestion



SCHEME OF CSF CIRCULATION
(Modified sagittal view)

Cranial Volume = Brain + CSF + Blood

VIIP <u>PROPOSED</u> PATHOPHYSIOLOGY

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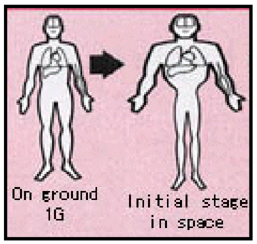

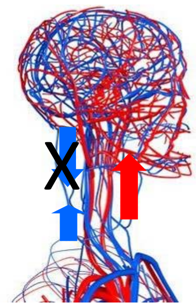

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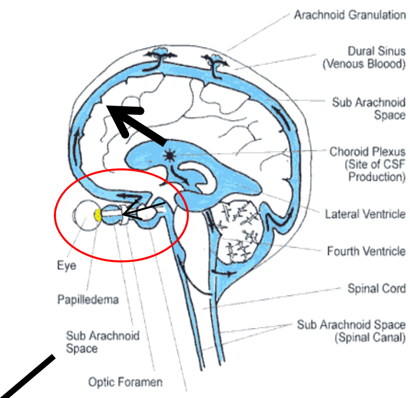

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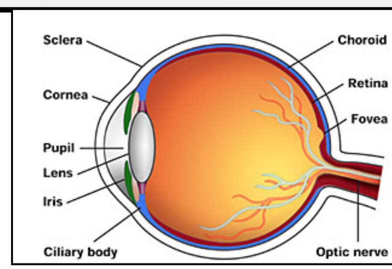
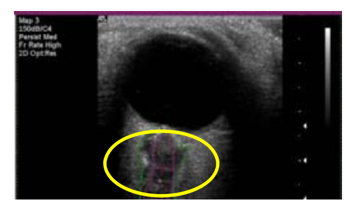
Up to +1.75 diopters



+ICP

Increased Optic Nerve Sheath Diameter



"Cotton wool" Spots - Altered blood flow

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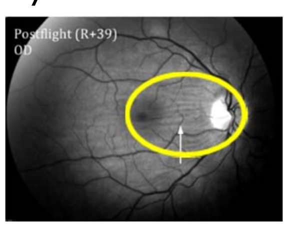
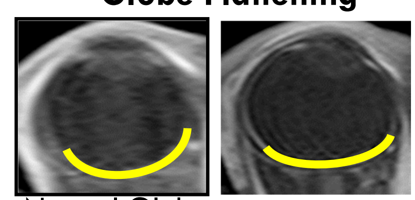
Ridges in back of eye



Optic Disc Edema
(Swelling)

Scotoma
Abnormal visual field

Globe Flattening



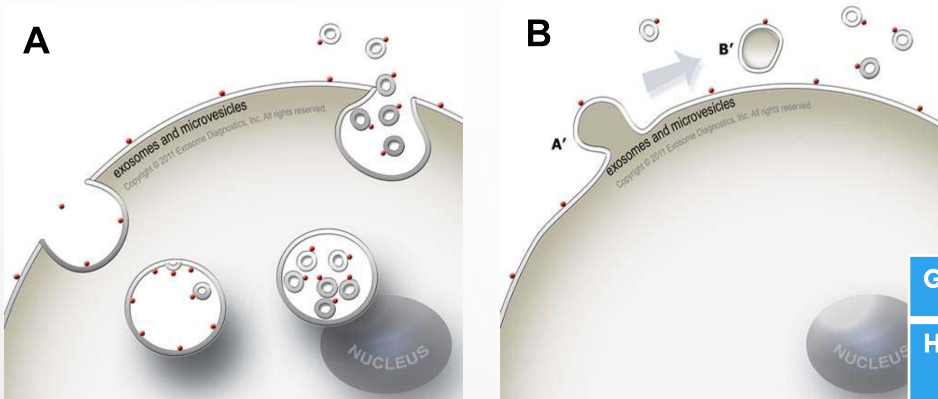
Normal Globe Flat Globe

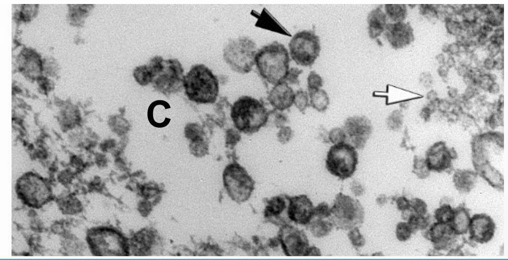
Courtesy Christian Otto, MD, HRP VIIP Project Scientist, July. 2014

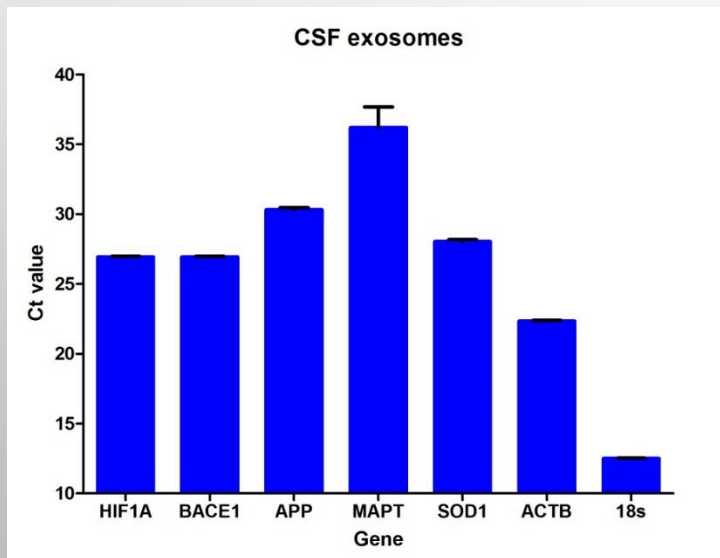
MRI Signs of Elevated ICP: IIH versus VIIP		
Imaging Assessment	Present in IIH (Specificity)	Seen in VIIP
Combined Stenosis Score	100%	Unknown
Flattening of Posterior Globes	100%	Yes
Tight Subarachnoid Space	100%	Unknown
Partially Empty Sella Turcica	95.3%	Yes
Optic Nerve Sheath Distension	88.4%	Yes
Optic Nerve Tortuosity	86%	Yes
Slit-like Ventricles	79.1%	Unknown

Comparison Table of MRI Findings in IIH Patients Compared to Those Identified in the VIIP Population

BRAIN GENE EXPRESSION SIGNATURES FROM CEREBROSPINAL FLUID EXOSOME RNA PROFILING (Zanello, USRA-Skog, ExosomeDx)



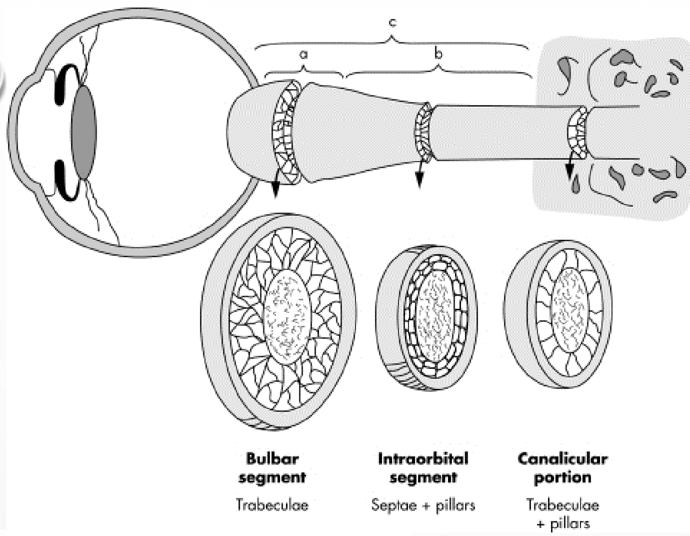




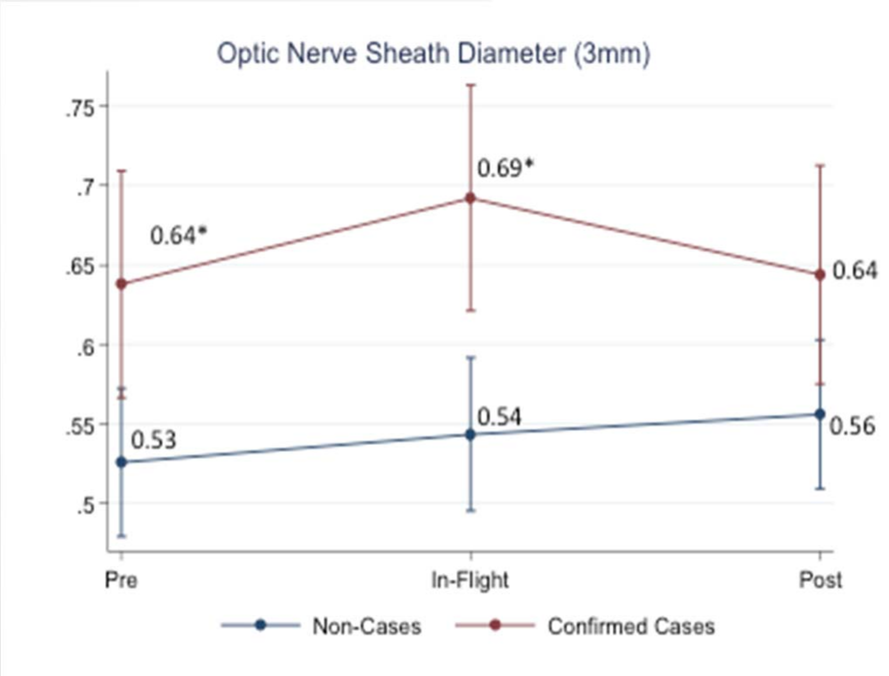
Gene
HIF1A (hypoxia-inducible factor 1)
BACE1 (beta-site amyloid precursor protein cleaving enzyme 1)
APP (amyloid precursor protein)
MAPT also known as Tau (microtubule associated protein)
SOD1 (superoxide dismutase 1)
ACTB (beta-actin)
18S rRNA (18S ribosomal RNA)

What do we know about the effects of spaceflight on the eye?

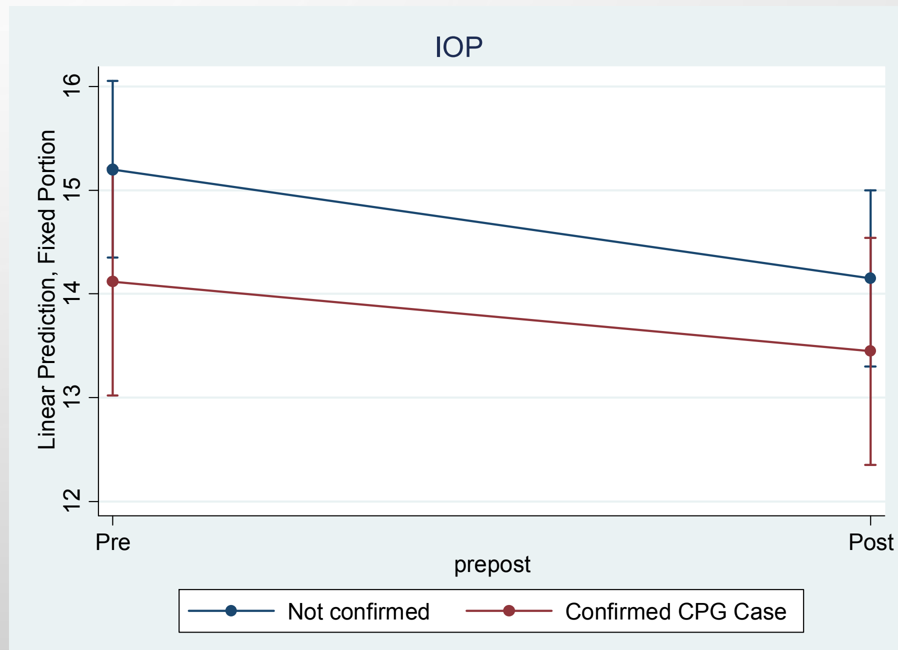
- *VIIP evidence*
- *Animal studies*



Schematic drawing of the optic nerve demonstrating the microanatomy of the ONS complex *(Killer et al, Br J Ophthalmol, 2003)*

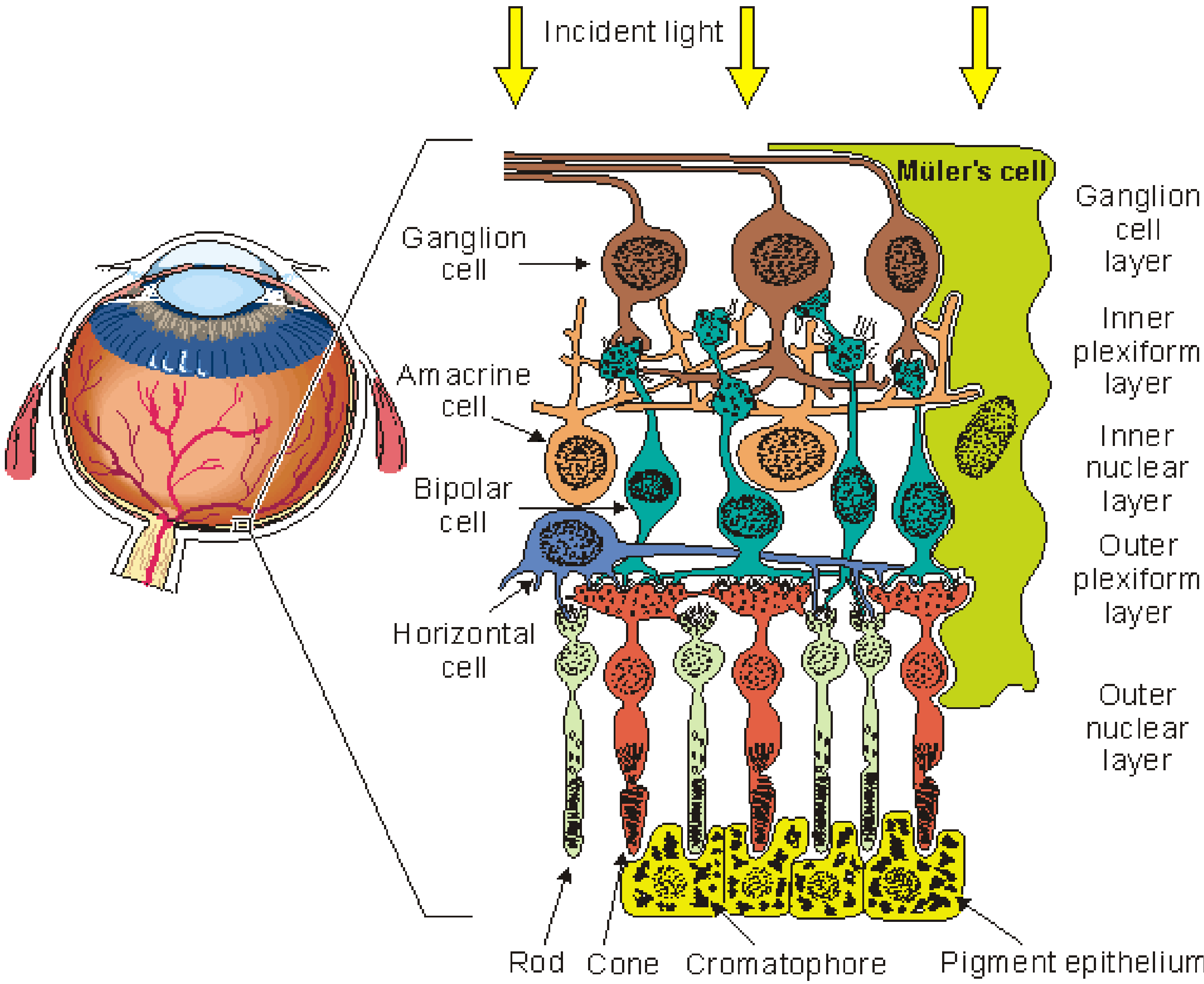


ONSD in astronauts: VIIP cases versus noncases preflight/in-flight/postflight (data from the NASA Lifetime Surveillance of Astronaut Health ***(LSAH)***.



Preflight and postflight IOP in VIIP cases and noncases.

VIIP Evidence Book-HRP 2012

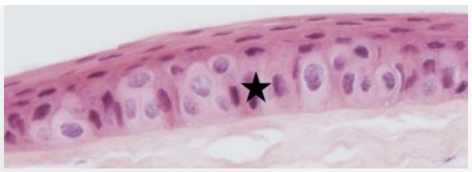


Incident light
Müler's cell
Ganglion cell
Amacrine cell
Bipolar cell
Horizontal cell
Ganglion cell layer
Inner plexiform layer
Inner nuclear layer
Outer plexiform layer
Outer nuclear layer
Rod
Cone
Cromatophore
Pigment epithelium

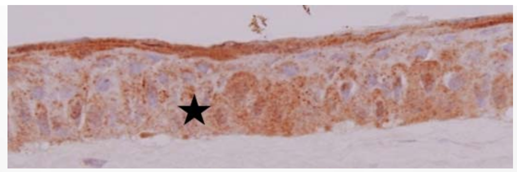
Spaceflight Effects and Molecular Responses in the Mouse Eye: Preliminary Observations After Shuttle Mission STS-133

Susana B. Zanello[1], Corey A. Theriot[2], Claudia Maria Prospero Ponce[3], and Patricia Chevez-Barrios[3,4]

[1] Division of Space Life Sciences, Universities Space Research Association, Houston, TX; [2] Wyle Science, Technology and Engineering, Houston, TX; Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, TX; [3] Pathology and Laboratory Medicine and Ophthalmology, Weill Medical College of Cornell University, The Methodist Hospital, Houston, TX; [4] Department of Pathology and Genomic Medicine, The Methodist Hospital, Houston, TX

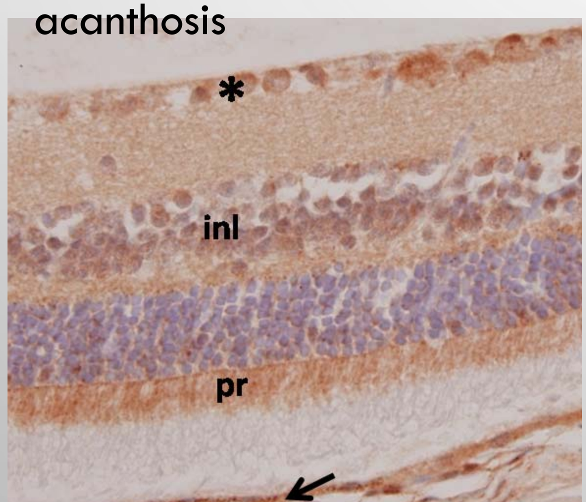


AEM R+7-Basal edema and acanthosis

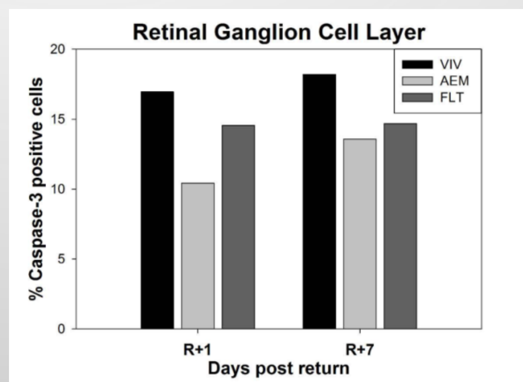


FLT R+1 - Caspase 3 positive

Retina

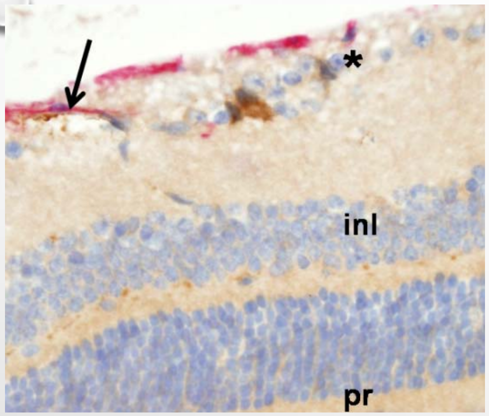


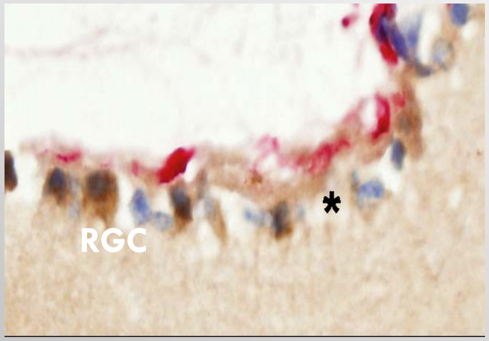
FLT R+1 Caspase-3 positive

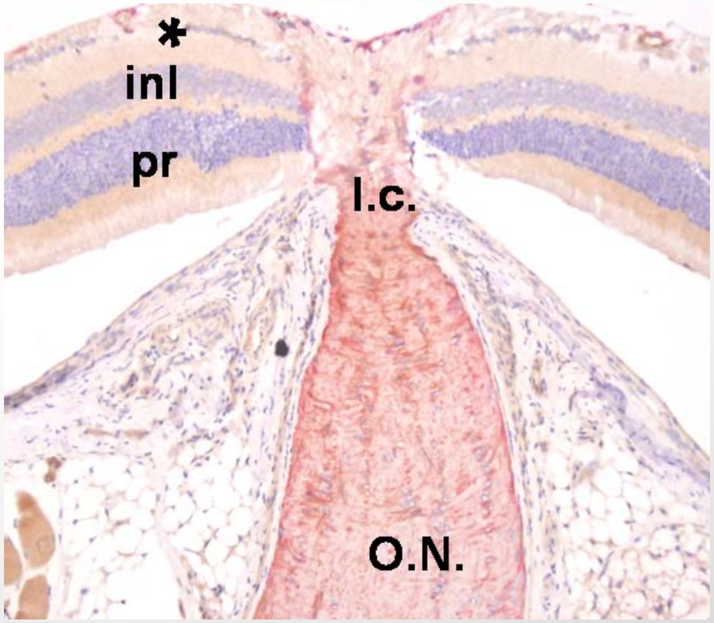


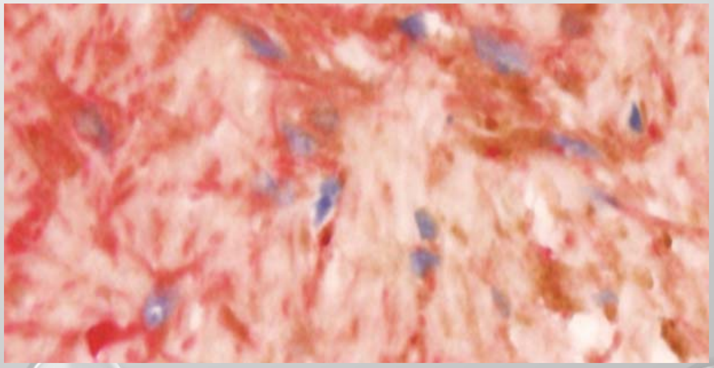
Gravitational and Space Research Volume 1 (1) Oct 2013 – 29

FLT R+1





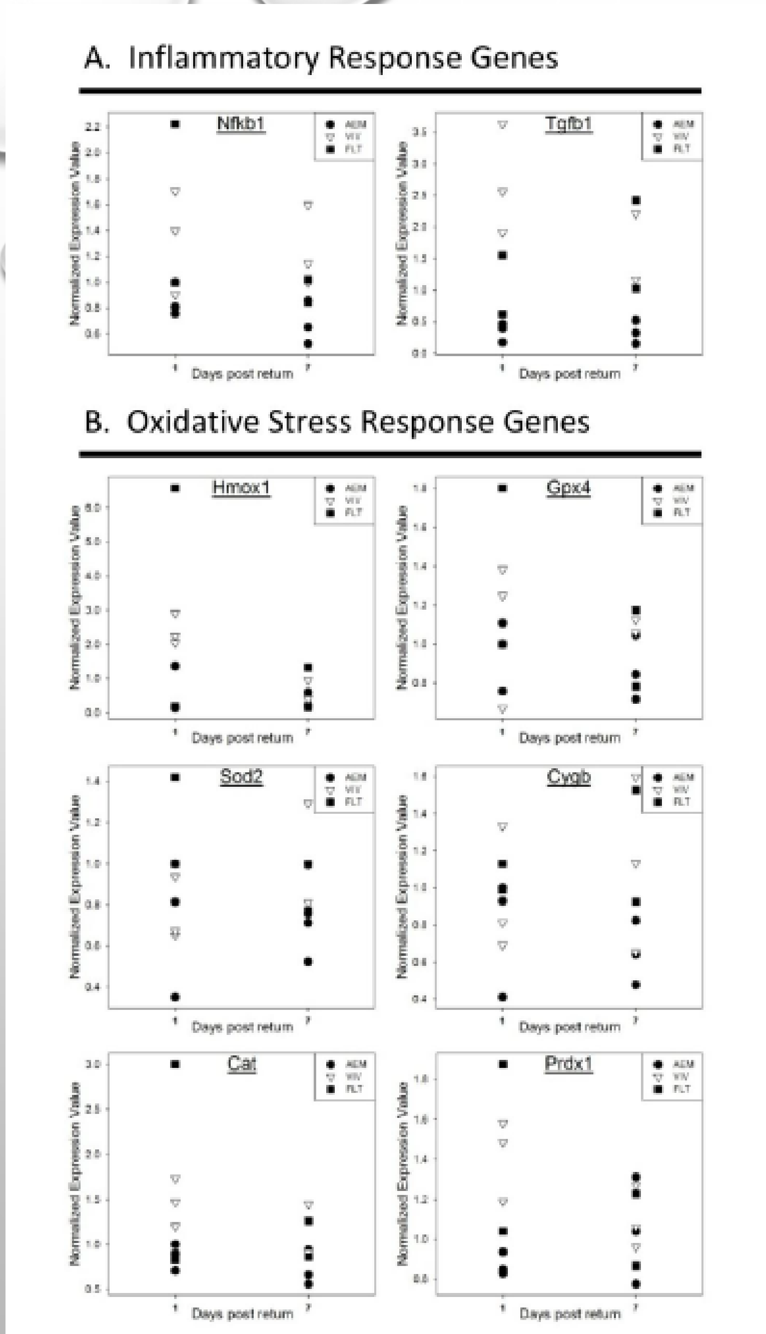


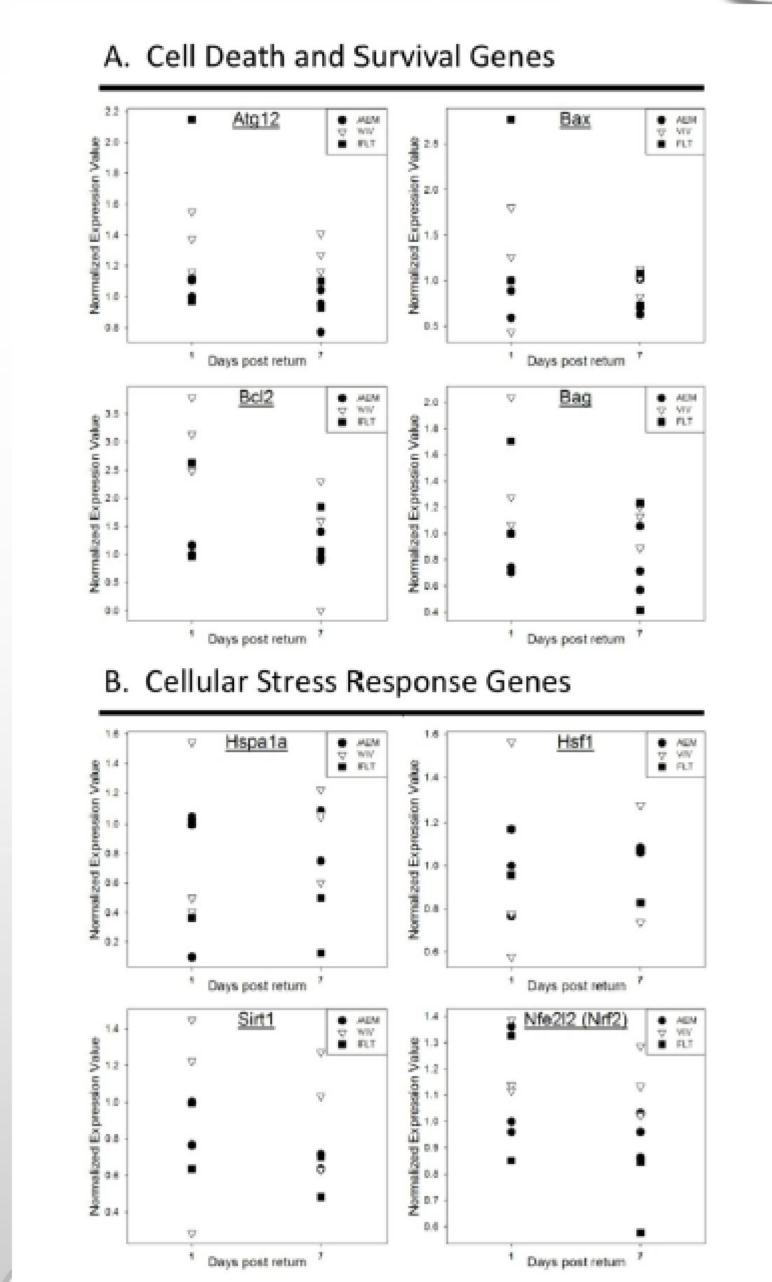


Post –laminar β-amyloid + region

GFAP: glial fibrillary acidic protein (glial activation)

β-amyloid (neuronal injury)


A. Inflammatory Response Genes
Nfkb1
Tgfb1
B. Oxidative Stress Response Genes
Hmox1
Gpx4
Sod2
Cygb
Cat
Prdx1
Normalized Expression Value
Days post return
AEM
VIV
FLT


A. Cell Death and Survival Genes
Atg12
Bax
Bcl2
Bag
B. Cellular Stress Response Genes
Hspa1a
Hsf1
Sirt1
Nfe2l2 (Nrf2)
Normalized Expression Value
Days post return
AEM
VIV
FLT

EXPERIMENTAL DESIGN

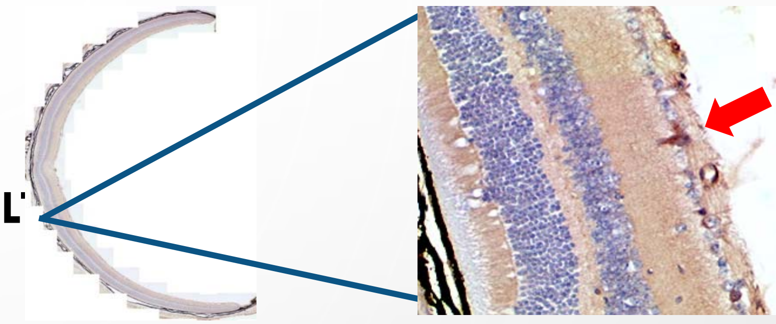
- **STS-135** C57BL/6 mice, 9-11 weeks old
  Conditions: AEM, flight
  Duration of flight: ~13 days
  Tissue collections: R+1

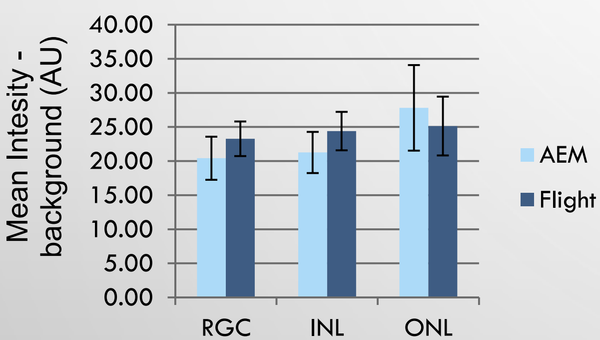
♀

RESULTS

- *Histological analysis for apoptosis by immunostaining*: 30 % more caspase-3 positive RGC in FLT vs AEM samples (n=3)

#52 FL





- *DNA damage caused by oxidative stress*, measured by densitometric analysis of the marker 8OHdG, was slightly more elevated in flight samples for the RGC and INL

PATHWAYS	• ER stress • Pyrimidine metabolism • Cytokine production and signaling • Sphingosine-1-P signaling
PROCESSES	• RNA processing (splicing) • Cell death of sensory neurons, RGC and microglia • Assembly of desmosomes
DISEASES	• Cancer • Neurodegeneration of nervous tissue • Degeneration of optic nerve • Reactivation of herpes virus

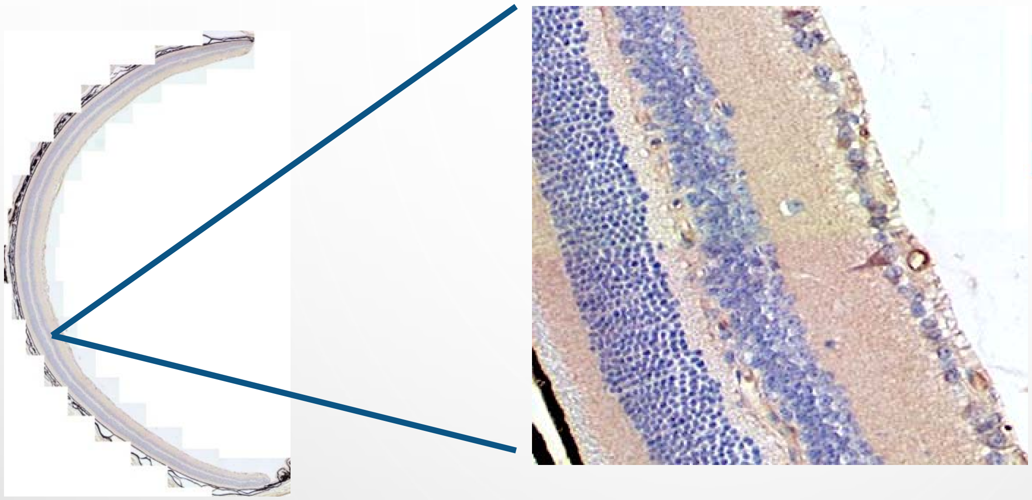
Gene expression profiling Flight vs AEM retina RNA:

- Affymetrix mouse expression array: 40,000 genes
- Differentially expressed genes: 139
- Pathway analysis by IPA bioinformatics software

STS-135 HISTOLOGY

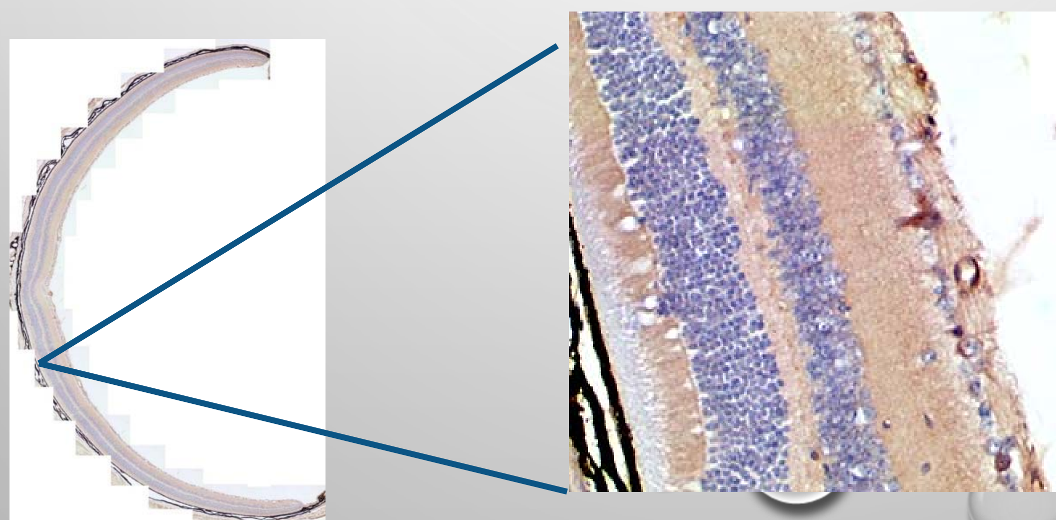
CASPASE-3 MEDIATED APOPTOSIS

#16 AEM



30 % more caspase-3 + RGC in FLT vs AEM (n=3)

#52 FLT

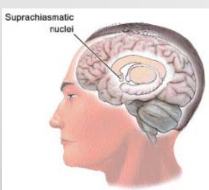


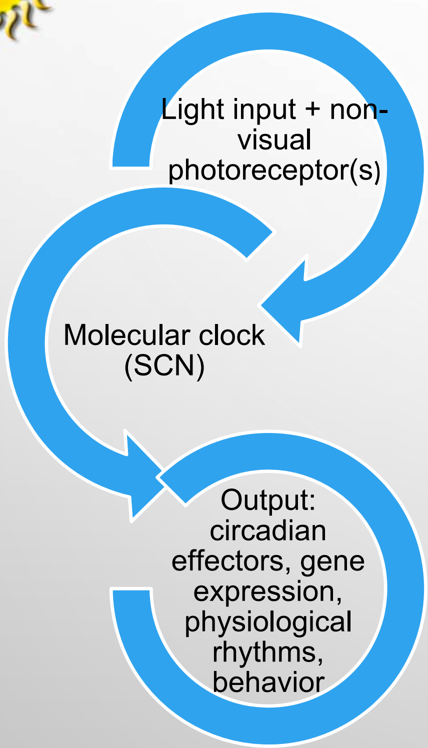
SHORT COMMUNICATION

Retinal Non-Visual Photoreception in Space

Aviat Space Environ Med 84(12):,1277-1280(4), 2013

Susana B. Zanello, Audrey Nguyen,
and Corey A. Theriot





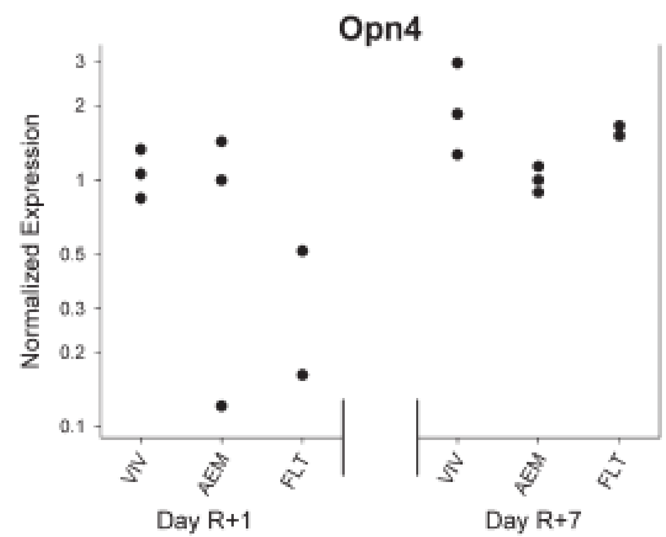
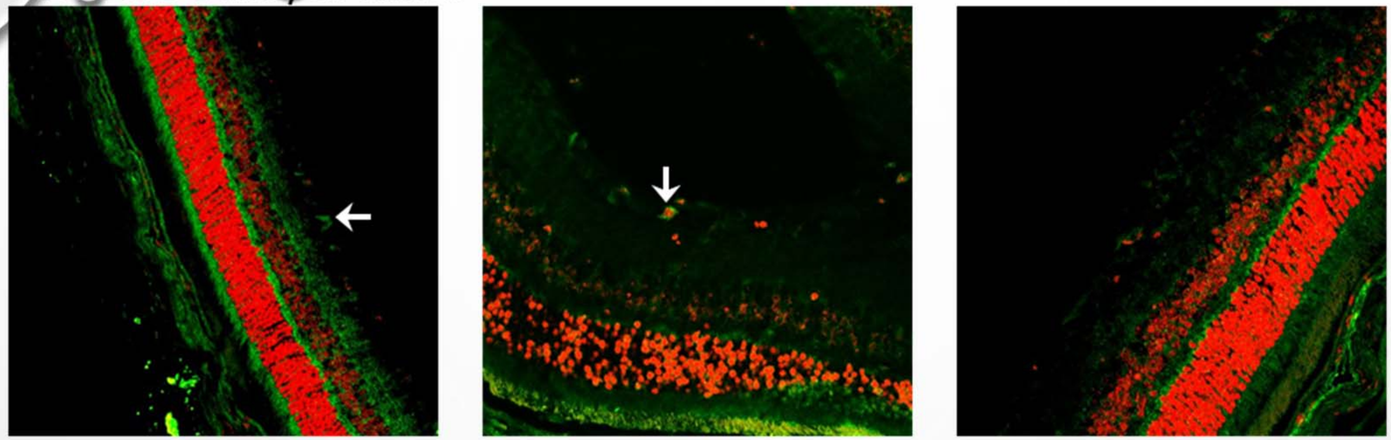


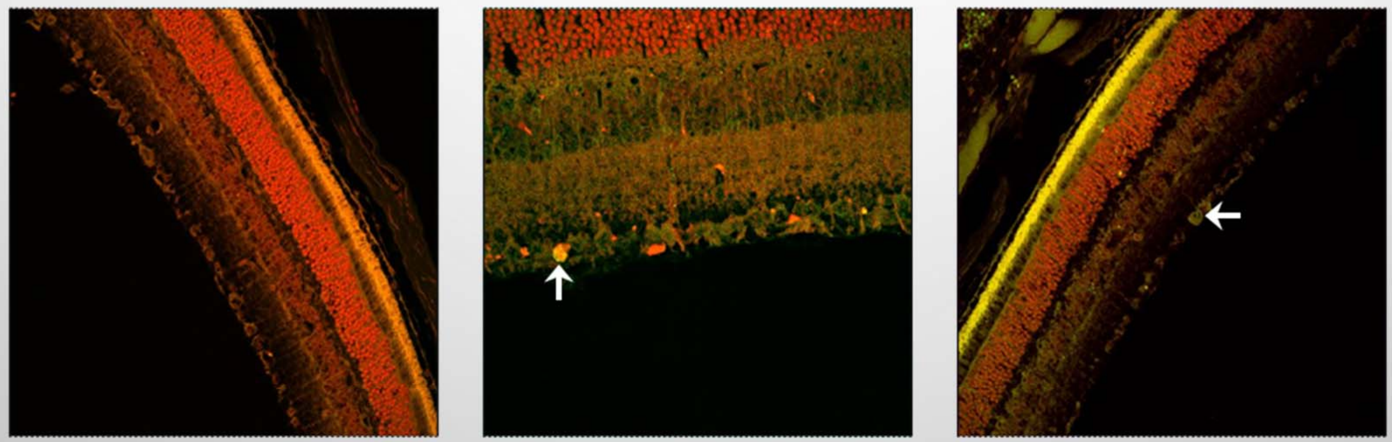
Fig. 1. Melanopsin (Opn4) gene expression levels in BALB/cJ mice retina samples from the STS133 experiment, measured by real time qPCR. Gene expression levels for vivarium control (VIV), animal enclosure module ground control (AEM), and flight (FLT) samples normalized to Hprt1 and graphed relative to an AEM control for days R+1 (left side) and R+7 (right side).

STS-133 Balb/cJ mice



VIV R+1 AEM R+1 FLT R+1

STS-135 C57BL mice



NEG CTRL AEM R+1 FLT R+1

Localization of melanopsin-positive ipRGC in mouse retina

Aviat Space Environ Med 84(12):,1277-1280(4), 2013

SUMMARY

THESE PRELIMINARY RESULTS SUGGEST THAT:

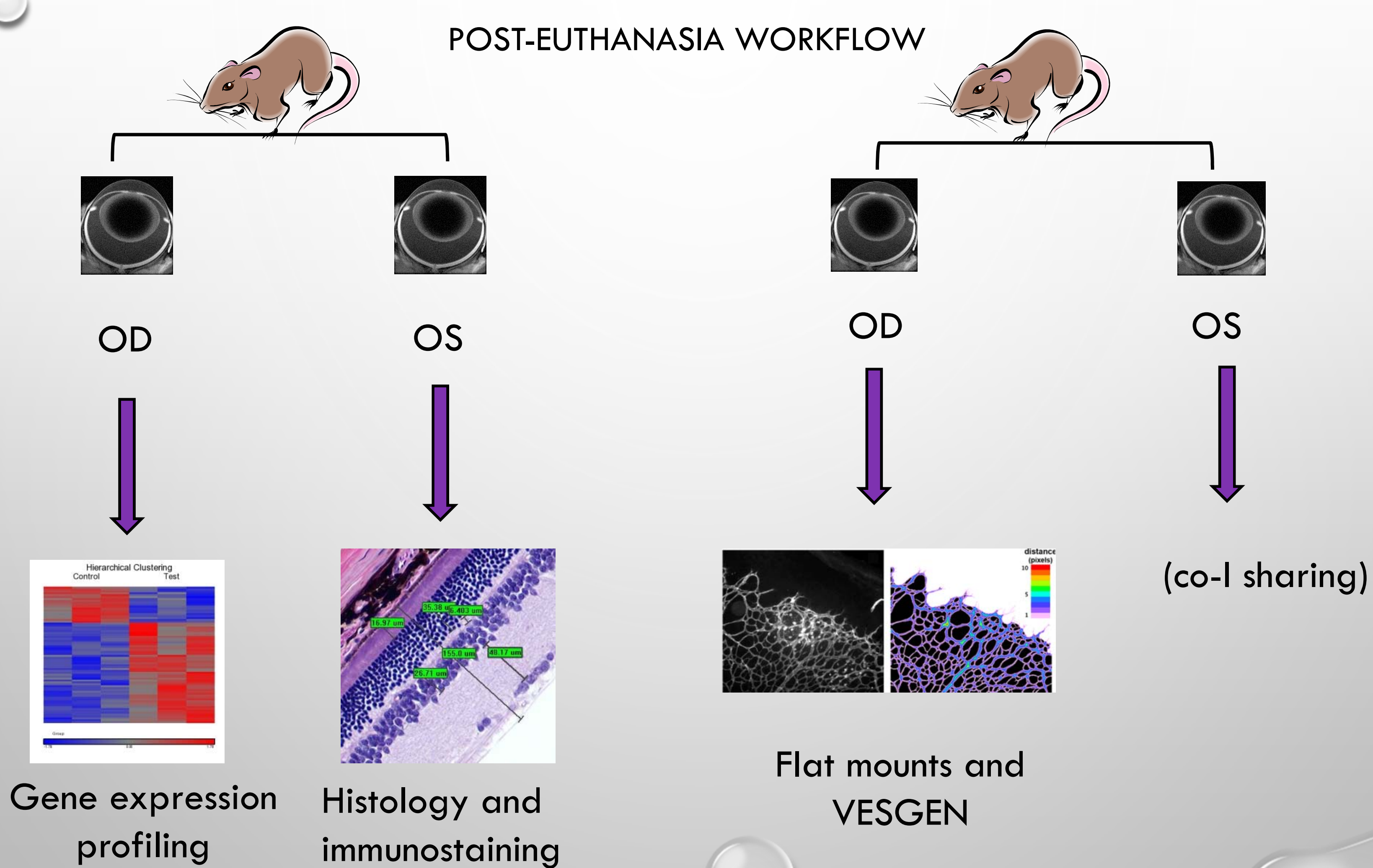
- OXIDATIVE STRESS AND NEURONAL LOSS OCCUR IN THE RETINA OF MICE EXPOSED TO SPACEFLIGHT
- DAMAGE IS PREFERENTIALLY LOCALIZED IN RGC
- OXIDATIVE AND CELLULAR STRESS IS REVERSIBLE TO SOME EXTENT UPON RETURN TO EARTH
- DAMAGE IS ALSO EVIDENCED BY GLIAL ACTIVATION AND NEURONAL/AXONAL INJURY
- ER STRESS AND NEURONAL/GLIAL CELL DEATH PATHWAYS ARE IMPLICATED IN NEURONAL CELL LOSS
- SUSCEPTIBILITY TO CELLULAR STRESS MAY AFFECT THE RESPONSE AND RESISTANCE TO THE EFFECTS OF SPACEFLIGHT IN THE RETINA AND THUS, THE SUSCEPTIBILITY TO FURTHER DAMAGE (DEGENERATION)
- MELANOPSIN EXPRESSION AND/OR SURVIVAL OF IPRGC MAY BE COMPROMISED UNDER THE STRESS OF SPACEFLIGHT CONDITIONS

GAPS (AGAIN)

- *VIIP1: WE DO NOT KNOW THE ETIOLOGICAL MECHANISMS AND CONTRIBUTING RISK FACTORS FOR OCULAR STRUCTURAL AND FUNCTIONAL CHANGES SEEN IN-FLIGHT AND POST-FLIGHT.*

- *VIIP12: WE DO NOT KNOW WHETHER GROUND-BASED ANALOGS AND/OR MODELS CAN SIMULATE THE SPACEFLIGHT-ASSOCIATED VIIP SYNDROME.*

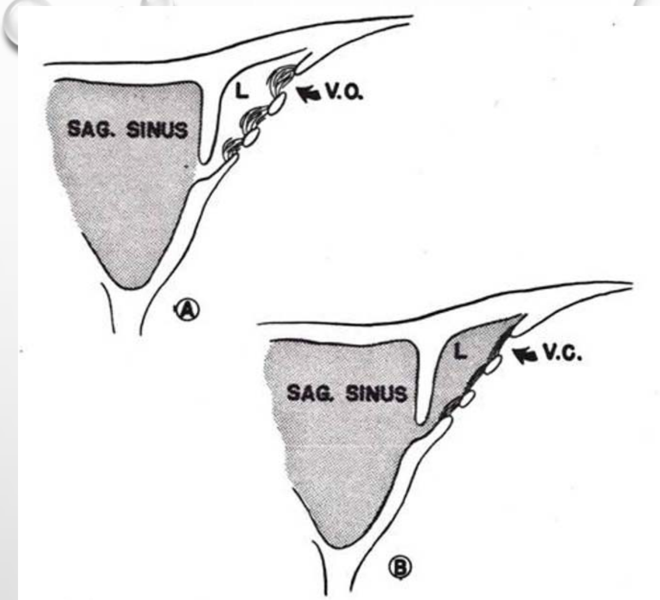
EVALUATION OF HINDLIMB SUSPENSION AS A MODEL TO STUDY OPHTHALMIC COMPLICATIONS IN MICROGRAVITY: OCULAR STRUCTURE/FUNCTION AND ASSOCIATION WITH INTRACRANIAL PRESSURE (Zanello, USRA, Chevez-Barrios, TMH, Vizzeri, U Pittsburgh, Parsons-Wingerter, NASA)



A GENE EXPRESSION AND HISTOLOGIC APPROACH TO THE STUDY OF CEREBROSPINAL FLUID PRODUCTION AND OUTFLOW IN HINDLIMB SUSPENDED RATS

Zanello, USRA; Chevez-Barrios, TMH; Rivera, TMH, Theriot, Wyle

A



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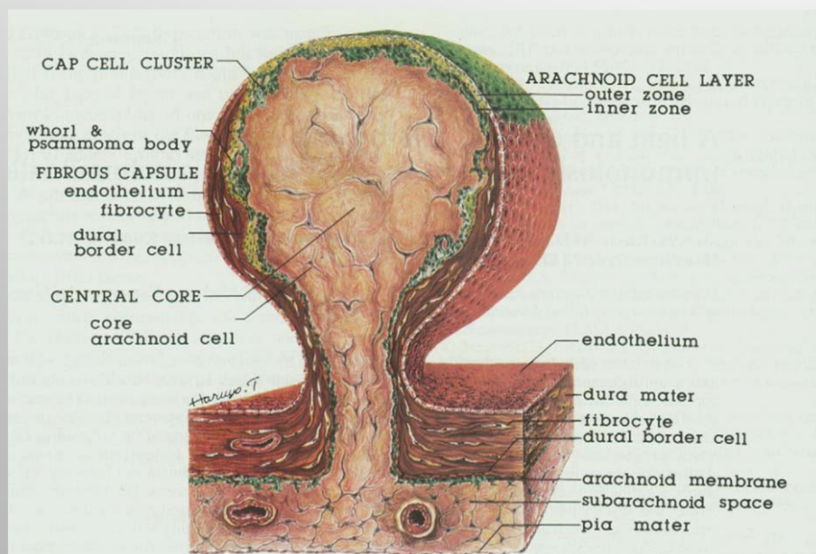

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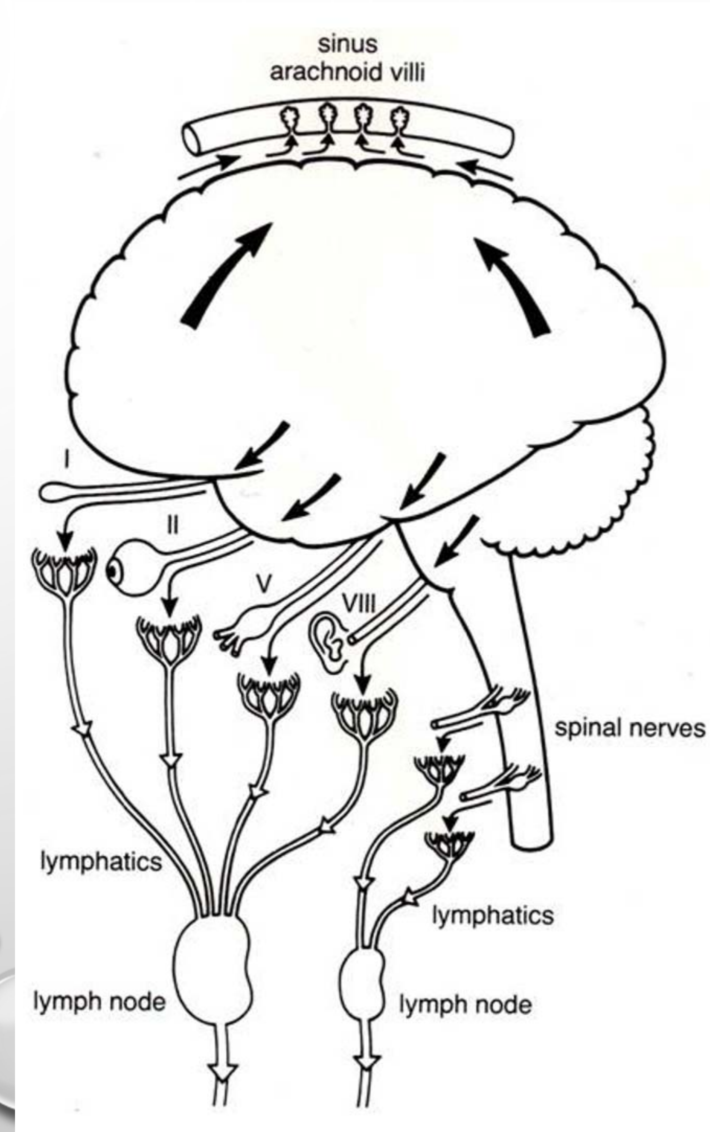
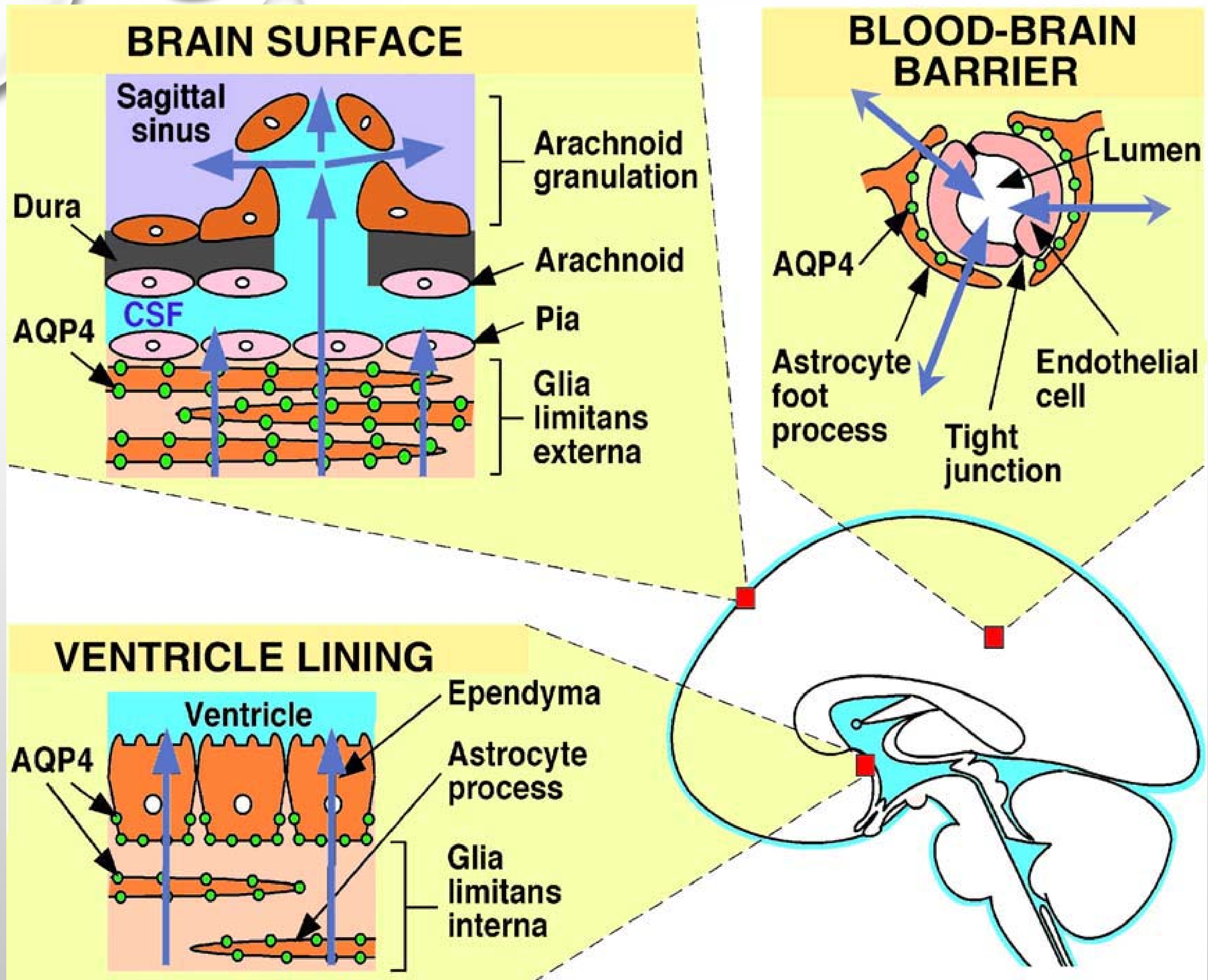
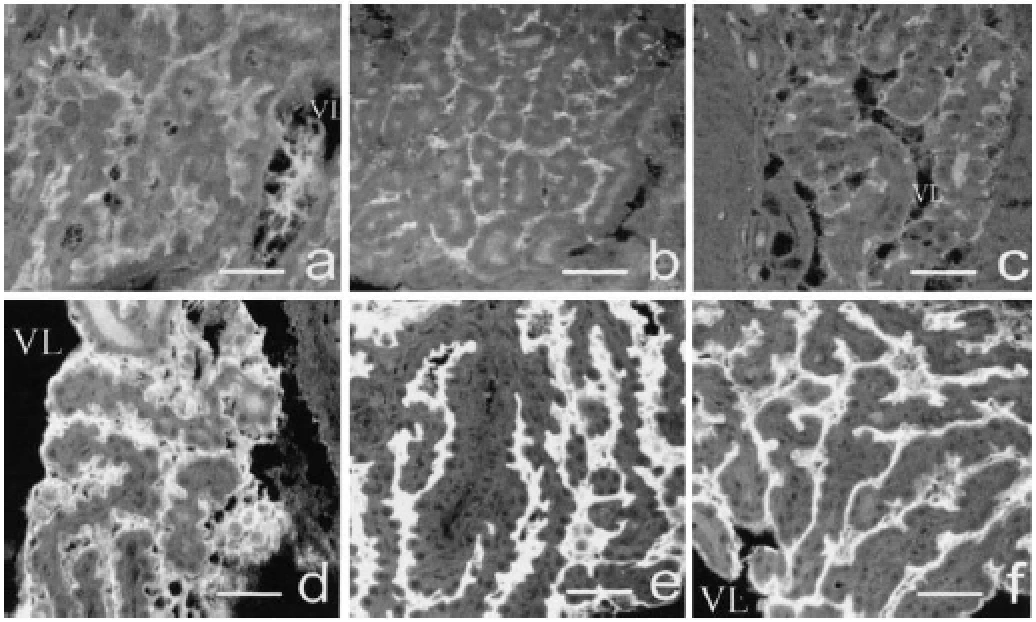


VIIP Evidence Book-HRP 2012



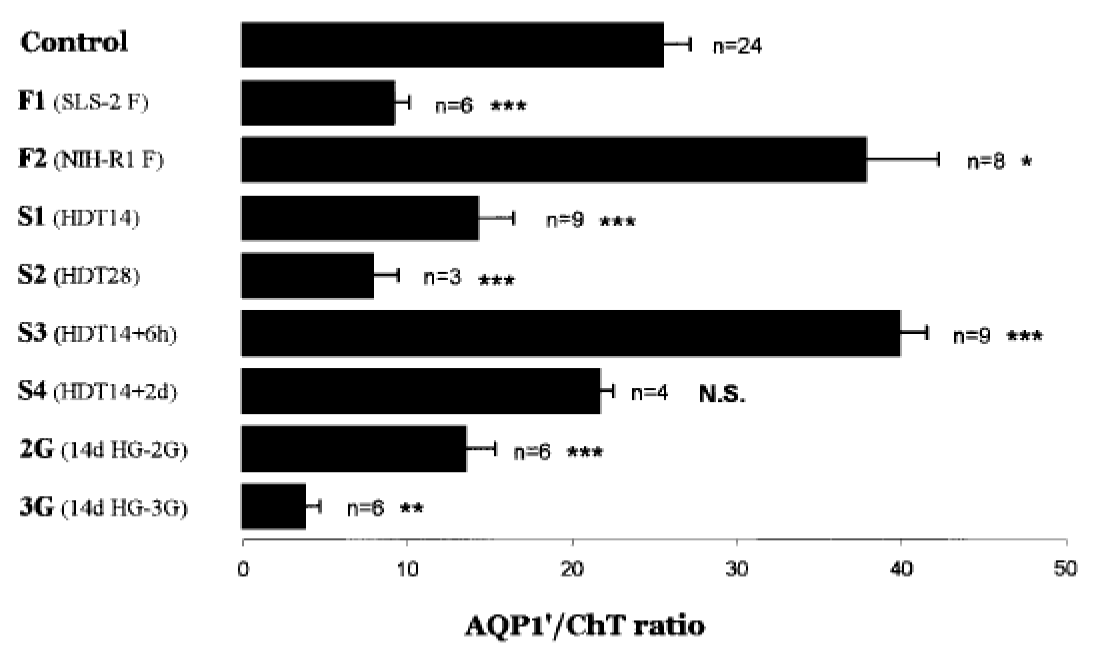
Schematic showing site of AQP4 expression in brain and three pathways for water movement out of brain in vasogenic edema (From: Verkman et al 2006, Biochim Biophyc Acta)

J. Appl. Physiol.
88: 843–850, 2000.



Altered gravity downregulates aquaporin-1 protein expression in choroid plexus

CHRISTOPHE MASSEGUIN,[1] MERYLEE CORCORAN,[2]
CAROLE CARCENAC,[1] NANCY G. DAUNTON,[2] ANTONIO GÜELL,[3]
ALAN S. VERKMAN,[4] AND JACQUELINE GABRION[1]
[1]*Institut des Neurosciences, Centre National de la Recherche Scientifique UMR 7624, Université Pierre et Marie Curie-Paris VI, Paris, France 75252;* [2]*Ames Research Center, National Aeronautics and Space Administration, Moffett Field, California 95034;* [3]*Centre National d'Etudes Spatiales (French Space Agency), Direction des Programmes, Paris, France 75001; and* [4]*Cardiovascular Research Institute, University of California, San Francisco, California 94143*

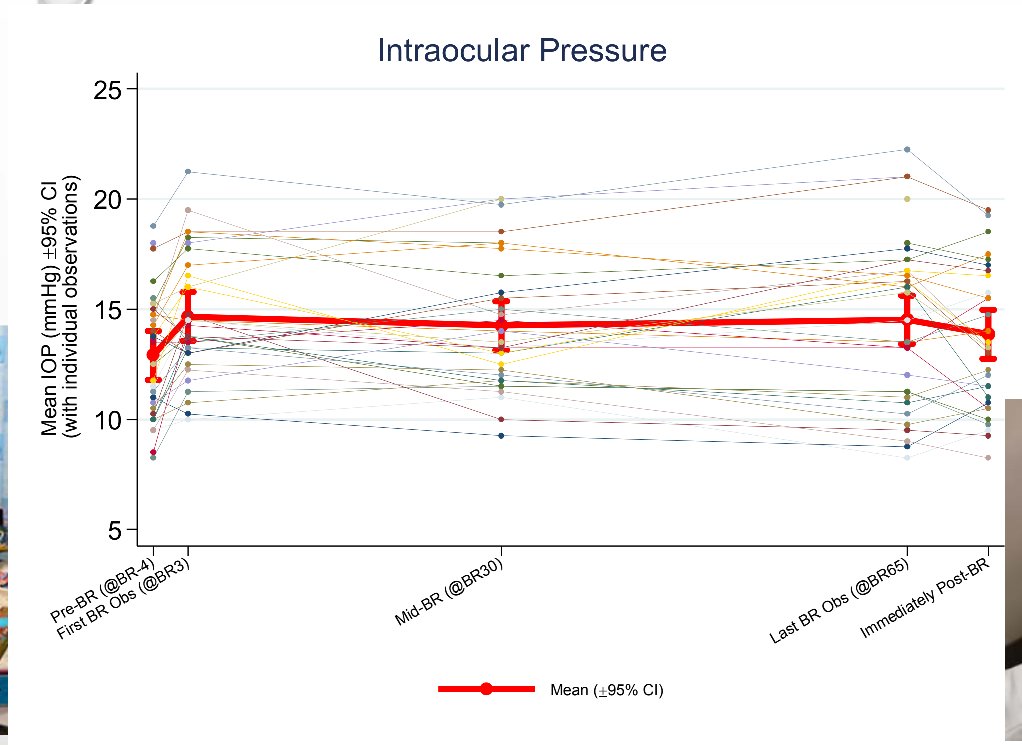


FLIGHT ANALOGS: Head Down Tilt Bed Rest

- Serves as a model for studying the physiological changes that occur during spaceflight under controlled conditions
  - Muscle Deconditioning - confinement to bed
  - Bone loss - long duration of inactivity
  - Fluid shifts - 6° head-down tilt
- Provides a platform for comparison between bed rest and space flight
- Provides a mechanism for testing countermeasures prior to being used in flight

Ocular testing schedule

	Pre-BR		BR			Post-BR	
	-11	-5	3, 10, 17, 24, 31	38	45, 52, 59, 66	+2	+9
Visual Acuity (Distance & Near)	•	•	•	•	•	•	•
Modified Amsler Grid	•	•	•	•	•	•	•
Red Dot Test	•	•	•	•	•	•	•
Color Vision	•	•	•	•	•	•	•
Confrontational Visual Field	•	•	•	•	•	•	•
Cycloplegic Refraction	•	•	•	•	•	•	•
IOP (Handheld)	•	•	•	•	•	•	•
IOP (Goldmann)	•	•				•	•
SD-OCT	•			•		•	•
Color Fundus Photography	•			•		•	•

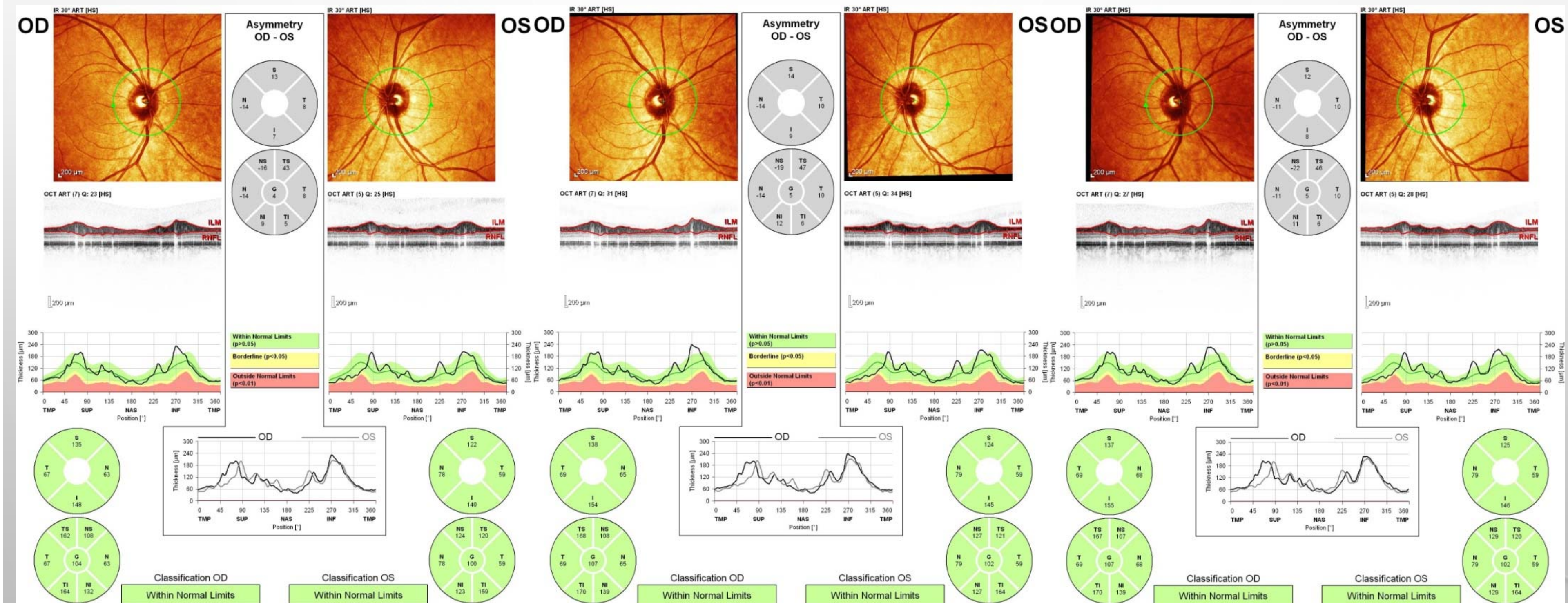


Source: NASA

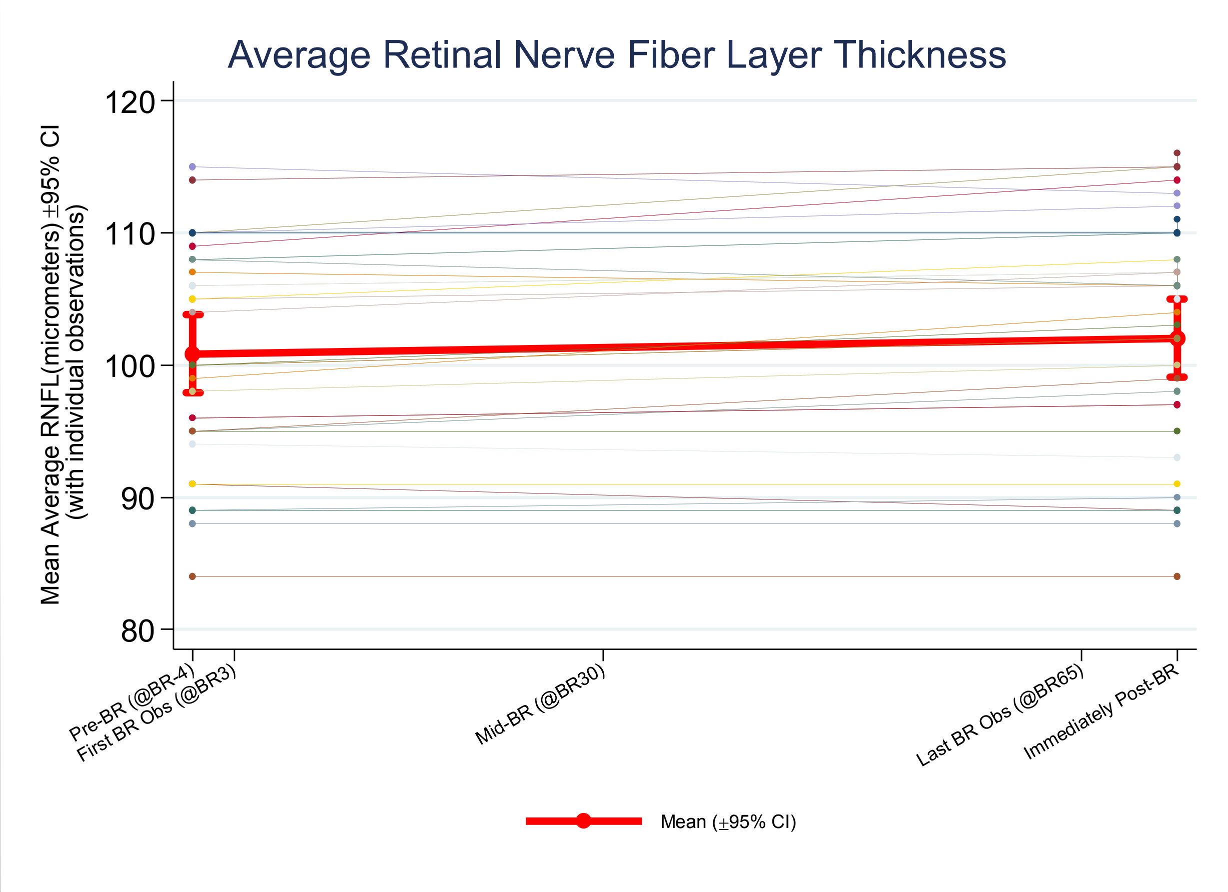
	IOP (mmHg)				
Day	Pre-BR	BR3	BR30	BR65	Post-BR
Mean	12.90	14.66	14.25	14.52	13.86
CI (+/- 95%)	(61.76, 64.24)	(62.86, 65.33)	(64.20, 66.67)	(64.80, 67.27)	(63.73, 66.20)
p (vs. pre-BR)	-	<0.001	<0.001	<0.001	<0.009

Spectralis OCT

- Optic Disc: Circular Scan



BR -12 **BR +2** **BR +8**



- Mean Average RNFL Thickness by Spectralis optical coherence tomography (OCT) significantly increased from a mean of 100.84μm in pre-BR to 102.03 μm in post-BR ($p < 0.001$).
- No in-bed measurements were taken for RNFL Thickness.

ACKNOWLEDGEMENTS

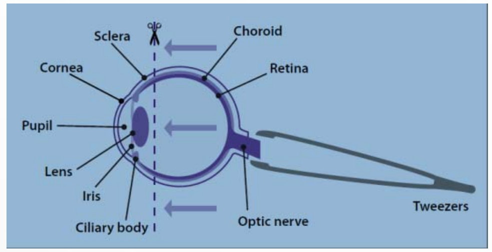
- Collaborators: Dr Chevez-Barrios, Dr Rivera (TMH), Dr Theriot (Wyle), Dr Fuller (UC Davis), Dr Skog (ExosomeDx), Dr Vizzeri, Dr Taibbi (UTMB/U Pittsburgh), Dr Ploutz-Snyder, Dr Fiedler (Biostatistics group, JSC)
- VIIP Science and Management Team: Jennifer Villarreal, Dr Christian Otto, Dr Yael Barr, Rachel Brady
- Flight Analogs Project (FAP) Science Team: Dr Ronita Cromwell, Dr Patrice Yarbough
- UTMB Flight Analog Project Research Unit
- NASA Human Research Program
- NASA JSC Biomedical Research and Environmental Sciences (SK) Division

QUESTIONS?

BACK-UP SLIDES

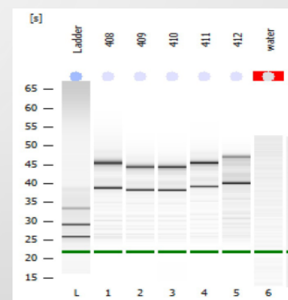
METHODS – GENE EXPRESSION

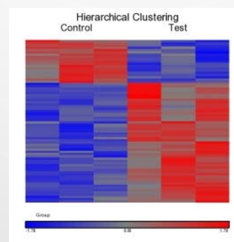
Eye globe
in
RNALater



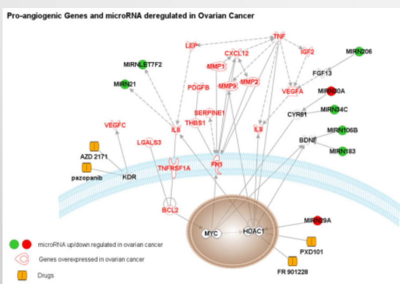
Retina in
RNALater

RNA isolation and QC (RIN)



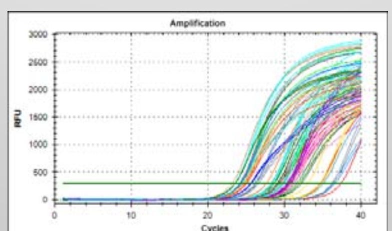


Microarray analysis



Pathway and processes
analysis

oxidative stress, hypoxia, microvascular remodeling and degeneration of various cell types (vascular, neural and glial cells)



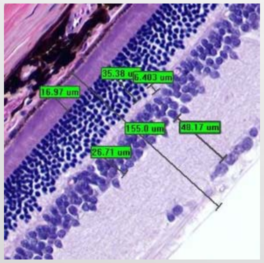
RT/qPCR

METHODS – HISTOLOGY

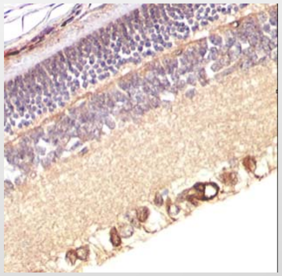
Eye enucleation and fixation in paraformaldehyde-based proprietary fixative

Paraffin embedding and sectioning

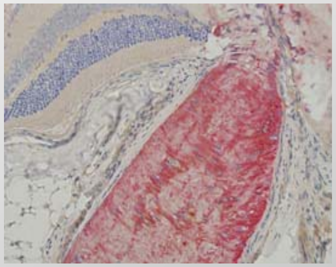
- H&E
- Pathology report
- Measurements



Specific markers (examples)



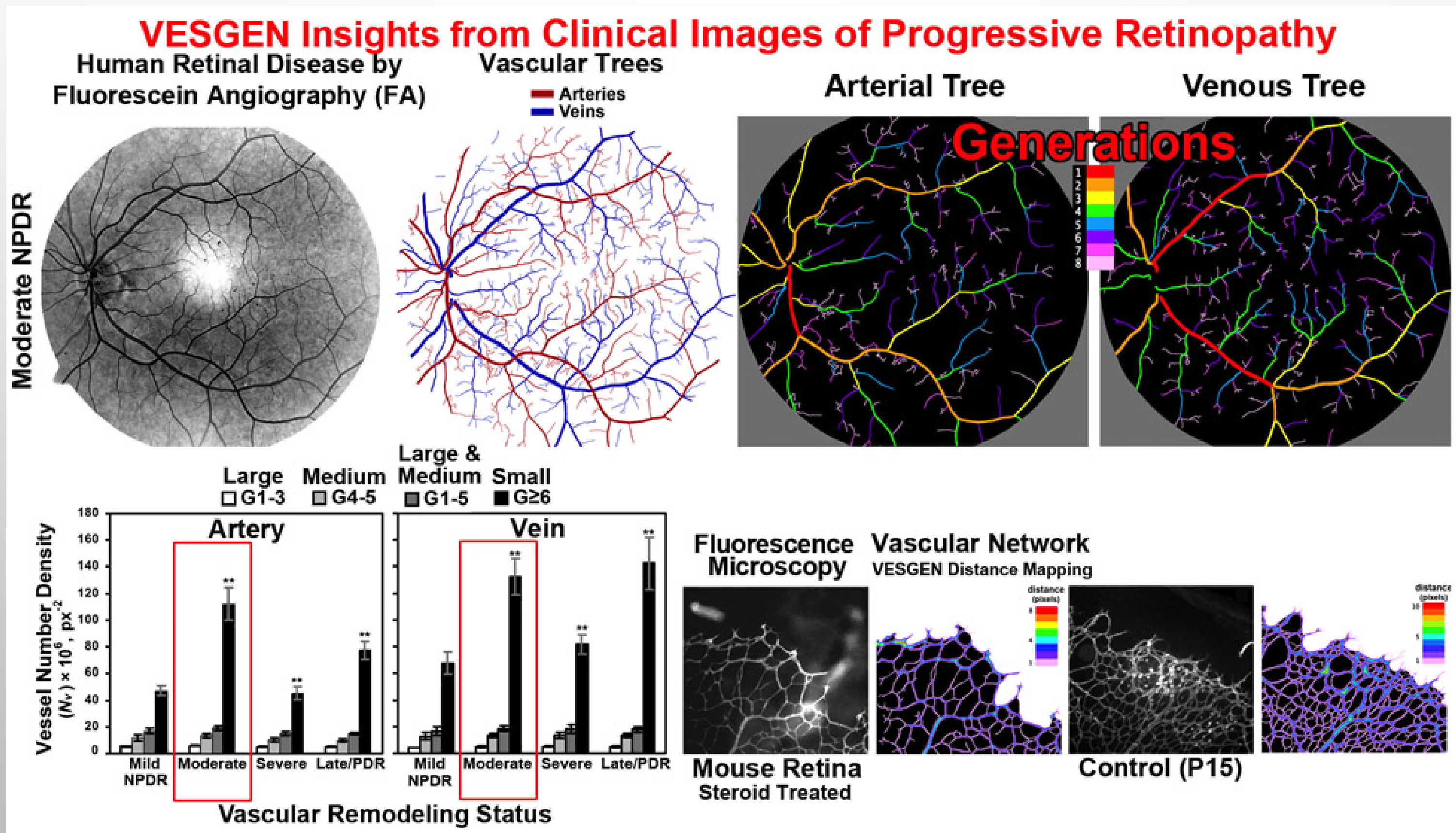
Apoptosis (caspase-3)



B-amyloid, GFAP

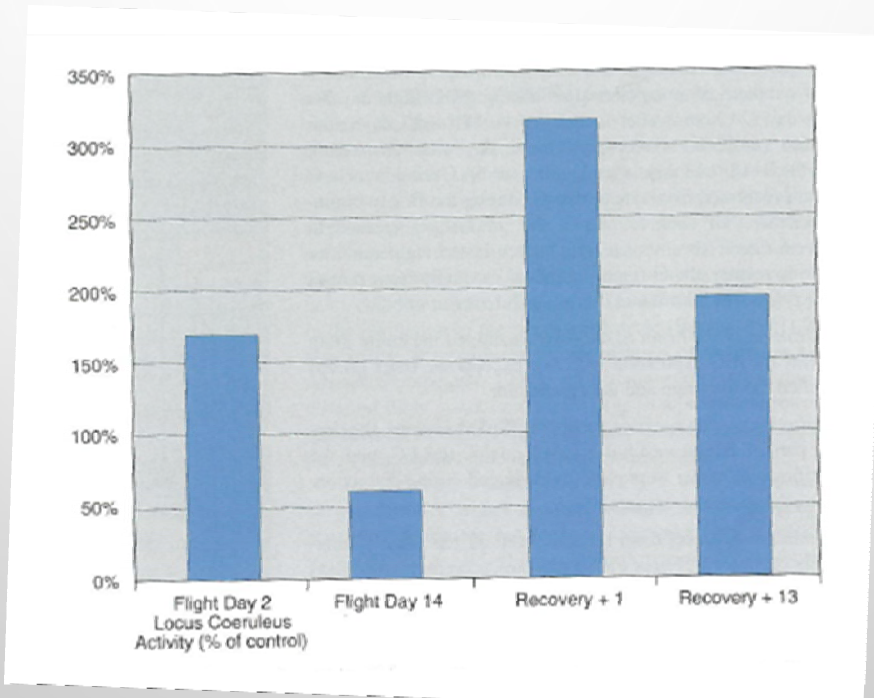
VESGEN

Eye enucleation and fixation in 4% PFA → Flat mounts → Isolectin staining of vessels + imaging

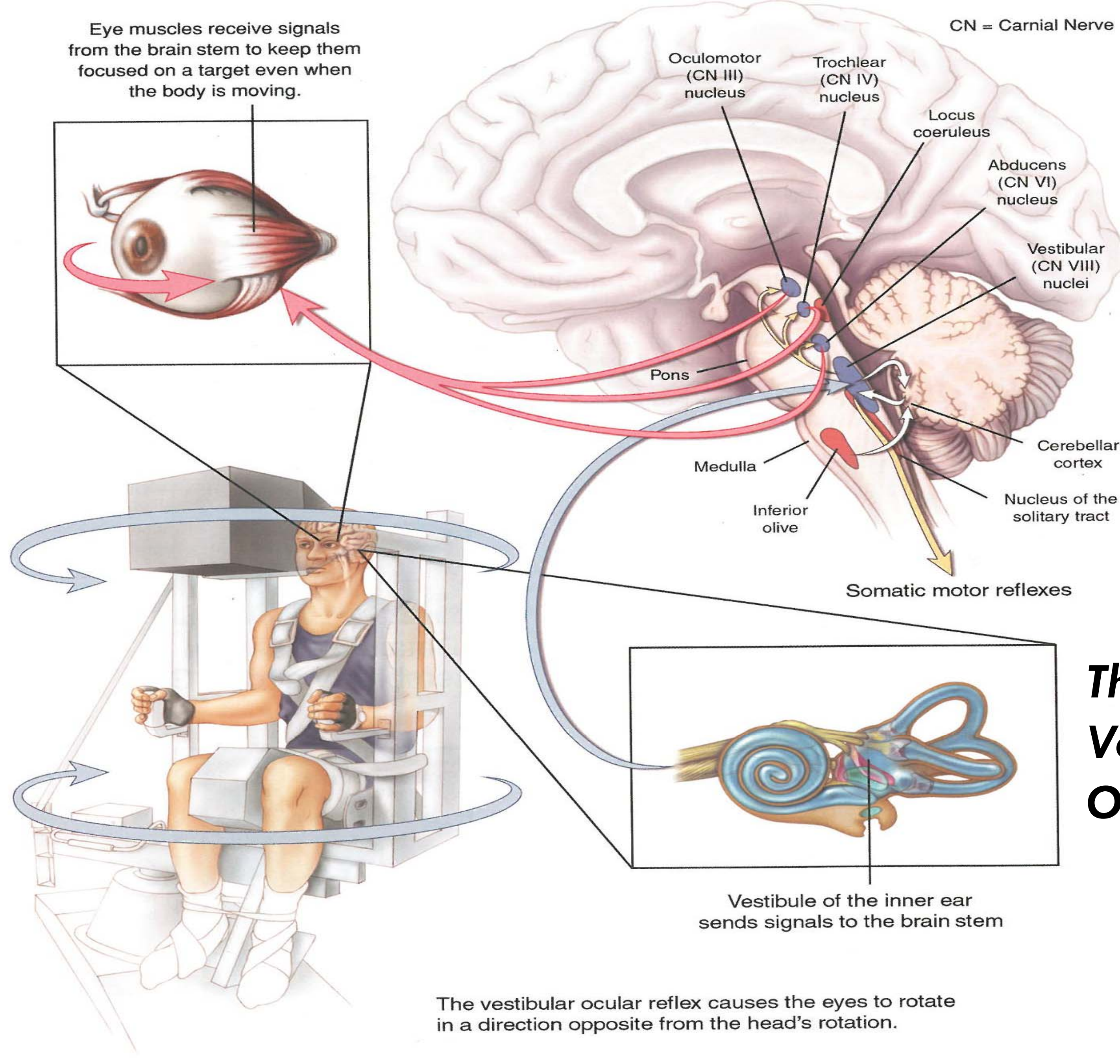


Compensation for these changes involve *plasticity*

- Changes in gene expression (e.g. immediate early genes like FRA, fos-related antigens) can evidence plasticity



Percentage change in FRA expression (# of immunoreactive cells) compared to ground controls in vestibular nucleus of male Fisher rats flown STS90 (Neurolab). Pompeiano et al, Acta otolaryngol 545:120-126, 2001)



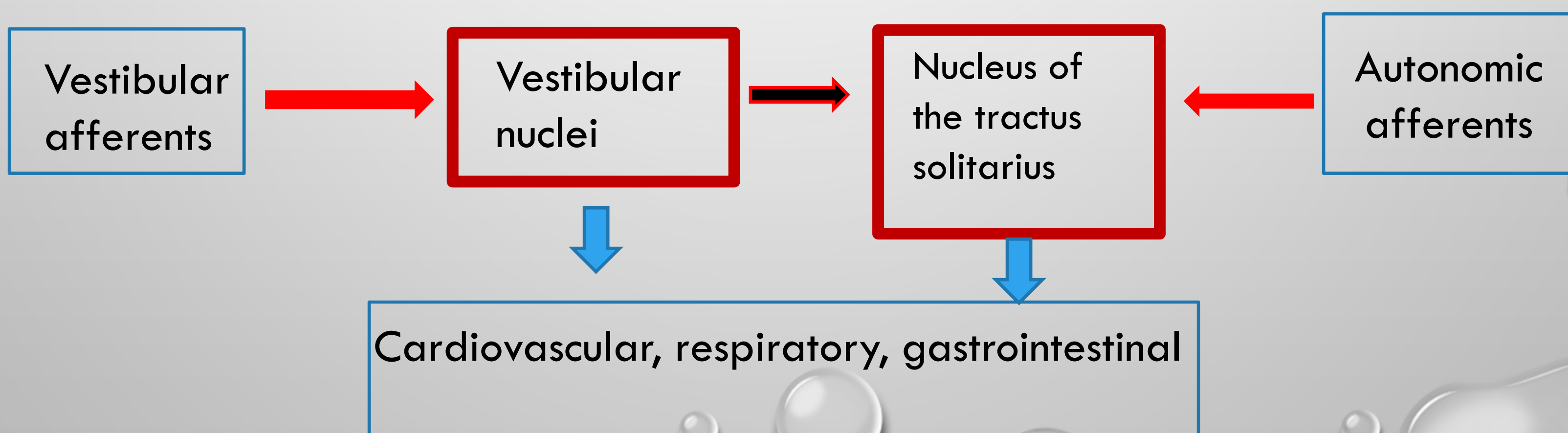
The Balance System: Vestibular Ocular Reflex

The vestibular system

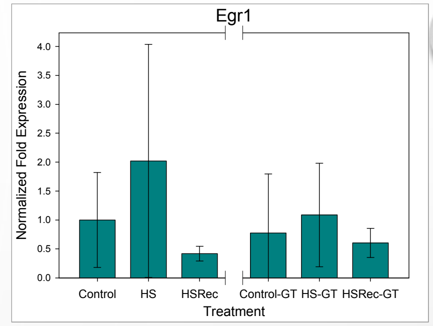
- senses the position and movement of the head in space
- controls the activity of the postural and eye muscles
- Influences the cardiovascular and respiratory systems
- Monitors body orientation

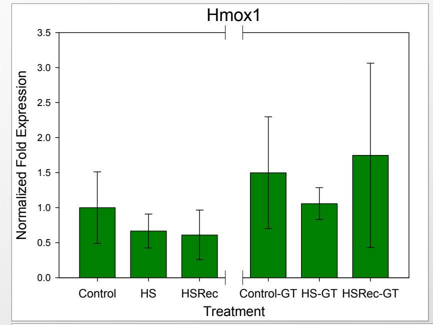
During spaceflight, astronauts show:

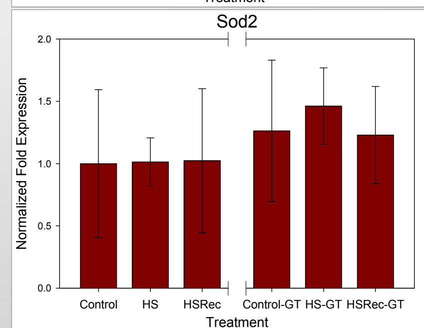
- Changes in balance and eye movements
- Alterations in the control of cardiovascular and respiratory activities
- Changes in body orientation and perception
- Sleep disturbances
- Balance disturbances after flight
- Ocular reflex (measured by ocular evoked myogenic potentials (oVEMPs) which represent extraocular muscle activity in response to vestibular stimulation are likely modulated by ICP. Jerin and Gurkov, Exp Brain Res. 2014 Jul;232(7):2273-9



INDUCTION OF EARLY GROWTH RESPONSE PROTEIN 1 AND HISTOLOGIC EVALUATION IN THE RETINA OF HINDLIMB SUSPENDED RATS (*submitted as a rapid communication to ASEM, under review*)







<u>Experimental design</u>: 34 wk old Brown Norway rats

Control diet

125 mg/kg resveratrol diet **(Rv)**

Epigallocatechin gallate (“**GT**” green tea extract diet)

- 2 weeks **HS**
- 2 weeks normal posture **control**
- 2 weeks HS + 2 weeks horizontal recovery **(HSRec)**

<u>Results:</u>

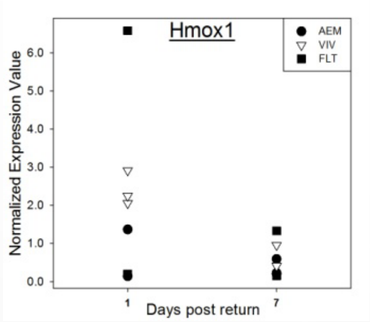
- Egr1 showed 2 fold increase in normalized expression values in retina from hindlimb suspension rats (HS) compared to normal posture controls (C-2wk). Egr1 expression returned to control levels upon 2 weeks recovery in normal posture after hindlimb unloading (HSRec).
- There was a suppression of the Egr1 induction by HS in HS rats fed a GT-rich diet (HS-GT)
- Compared to control diet, the GT enriched animals showed upregulation of the antioxidant enzymes Hmox1 and Sod2.
- Total retinal thickness was increased in retinas from resveratrol-fed HS animals compared to control diet-HS animals.

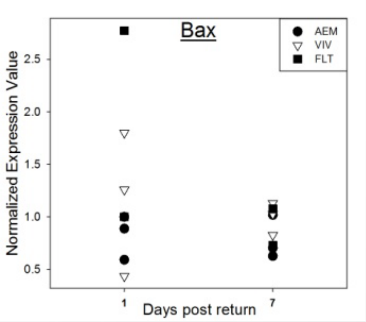
Total Retina	**Mean (µm)**	**p value**
HS	107.20	
HS-Rv	129.88	0.049
HSRec	107.90	
HSRec-Rv	108.89	

STS-133 AND STS 135 GENE EXPRESSION

STS 133

Real time qPCR





Oxidative stress **Cellular death**

STS 135

- Microarray processing and analysis performed at the UTMB Genomics Core Laboratory (n=3)
- Affymetrix mouse expression array: 40,000genes
- Differentially expressed genes: 139
- Ingenuity systems iReport generated