Effects of Extreme Hypobaric Environments upon the Brain in Specialized Operators

15 Nov 2018

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Integrity ★ Service ★ Excellence

Cleared, SAF/PA, Case # 2018-0546, 25 Sep 2018.

Disclosure Information

- **No financial relationships to disclose**
- **No discussion of off-label use and/or investigational use in my presentation**
- **The views expressed are those of the authors and do not necessarily reflect the official policy or position of the Air Force, the Department of Defense, or the U.S. Government**

Radiologist/Neuroradiologist
Former Senior Flight Surgeon

U.S. Air Force photo by A1C Zade C. Vadnais

Overview

- **U-2 pilot, physiologist, and normative database study *(2011-2014)***
  - **105 U-2 pilots, 89 aerospace physiology chamber inside observers (AOP), 148 controls**
- **NASA astronaut study *(2015)***
  - **39 astronauts**
- **Single hypobaric exposure study *(2014-2017)***
  - **96 Aircrew Fundamentals Course (AFC) trainees, 65 controls, 14 AOP**
  - **"Duration of Effects" follow on study *(2018-2019)***
- **Swine studies *(2015-present)***
- **Summary**

Background

- U-2 Dragon Lady operates in an extreme environment
- Astronaut and U2 crew protection based on years of experience and research
- U-2 pilots and astronauts during EVAs experience a hypobaric environment of approximately 4.3 psia (approximately 30,000 ft/9144 m)
- 300% (2006-2010) increase in neurological decompression sickness in U-2 pilots led to neurological evaluation including brain imaging

Distribution A: Approved for public release; distribution is unlimited. Case Number: 88ABW -2014-5678, 10 Dec 2014

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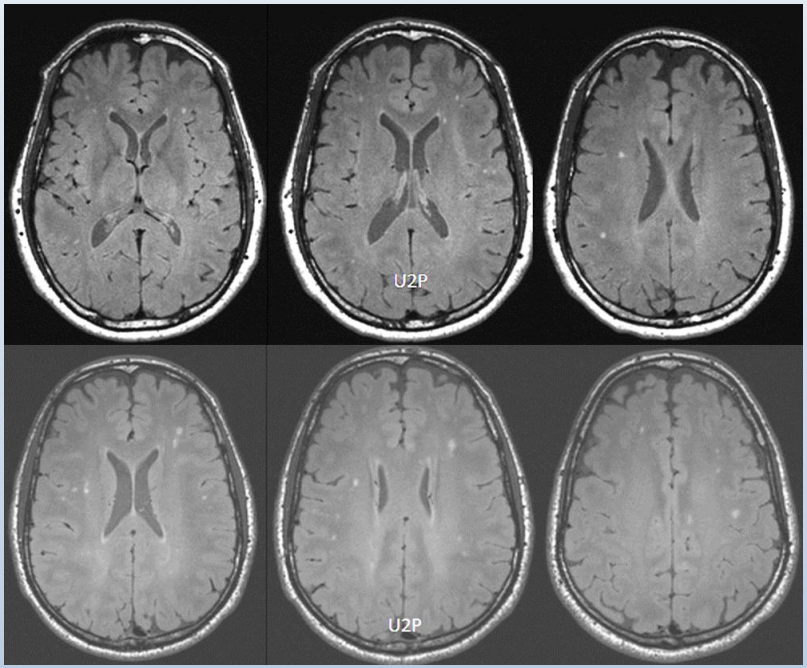
U-2 Study – Repetitive Exposure

- Imaging began as part of evaluation for Neurologic DCS episodes - 2011
- Focal punctate white matter hyperintensities (WMH) on FLAIR MRI
- MRI highly reproducible



U2P and AOP, with or without NDCS

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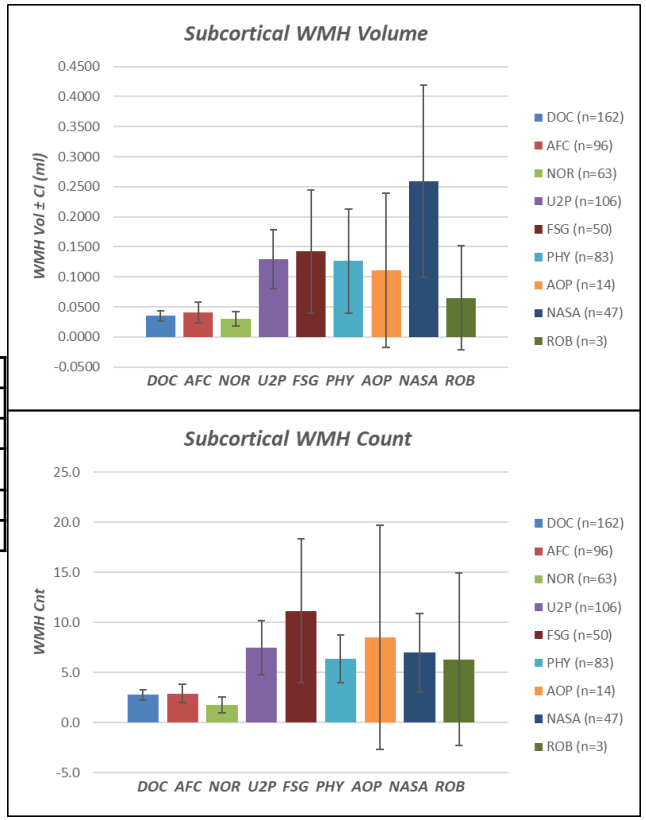
Phase 1 Repetitive Exposure White Matter Hyperintensities

- Significantly increased subcortical WMH volume/count in U2P & AOP/PHY
- AFC ≈ DOC ≈ NOR
- U2P ≈ AOP/PHY ≈ FSG
  - Individual variability
- Volume most clinically significant

	DOC	U2P	PHY
WMH vol (mean±CI)	0.035±0.009	0.129±0.049	0.126±0.086
WMH cnt	2.8±0.5	7.5±2.7	6.4±2.4
Mann-Whitney-Wilcoxon	DOC:PHY	DOC:U2P	U2P:PHY
WMH volume (mL)	p=0.0287	p<0.0001	p=0.4046
WMH cnt	p=0.0499	p=0.0374	p=0.9388

DOC – doctorate controls
U2P – U-2 pilots
AOP/PHY – aerospace operational physiologists
AFC – aircrew fundamental course students
NOR – combat arms students
FSG – flight surgeons
NASA – astronauts
ROB – reduced oxygen breathing device



McGuire et al. Neurology 2013;81:729-735
McGuire et al. Ann Neurol 2014;76:719-726

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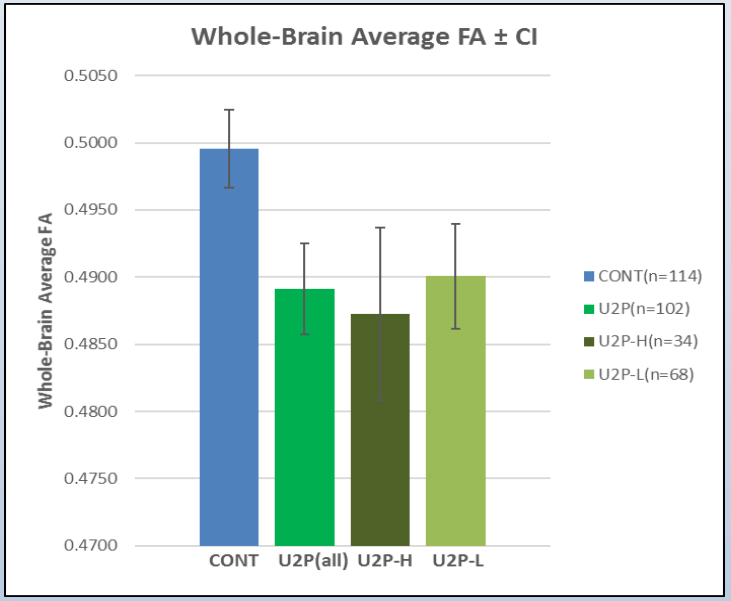
Phase 1 Repetitive Exposure Fractional Anisotropy

- **Whole brain average FA assesses entire WM**
  - **FA believed to correlate with axonal integrity**
  - **Used ENIGMA-DTI protocol to exclude visible areas of WM injury (punctate WMH)**
  - **KS p<0.001; GLM p<0.001**
    - **Kolmogorov-Smirnov (KS)**
    - **Generalized linear model (GLM) with age as nuisance covariate**
- **Reflects ~ 2% decline in axonal integrity**
- **Decline in axonal integrity appears to track with WMH burden**
- **Results contingent upon cross calibration of scanners**
  - ***46 subjs dual imaged (r=0.85; COV=4%). UT and Wilford Hall magnets**



McGuire et al. Aerosp Med Hum Perform. 2016.

Repetitive Exposure Neurocognitive Differences

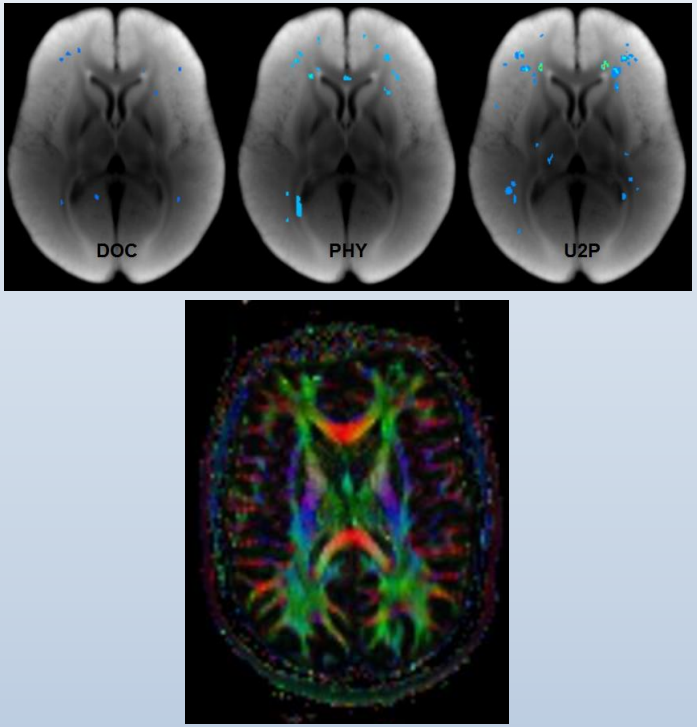
- Significant decrease in current computer-based Microcog testing in U2P compared to AF pilot controls
- Pattern of change similar to all other neurological diseases with subcortical injury; no change in IQ
- Multiple indices indicate pilots are similar at undergrad pilot training
- Decrease suggests diffuse WM process; MicroCog absolute values generally decreased with greater WMH burden within the U2P population

	MicroCog	U2P (n=93)	AFP (n=80)	t-test (2-tailed) Significance	Sidak (2-tailed) Significance
1	Attention/mental control	104.4	103.8	p=0.696	p=0.997
1	Reasoning/calculation	99.4	106.5	p<0.001	p=0.001
1	Memory	105.5	110.9	p=0.007	p=0.036
1	Spatial processing	109.1	109.1	p=0.989	p=1.000
1	Reaction time	107.3	104.8	p=0.047	p=0.216
2	Information processing speed	103.6	106.5	p=0.100	p=0.189
2	Information processing accuracy	102.1	105.8	p=0.016	p=0.032
3	General cognitive functioning	103.5	108.5	p=0.002	p=0.004
3	General cognitive proficiency	105.4	108.6	p=0.037	p=0.072

McGuire et al. Neurology 2014;83:638-645

U-2 Study – Summary

- **Recurrent exposure to nonhypoxic extreme hypobaria incites:**
  - **Focal punctate WMH on MRI**
  - **Diffuse decrement in axonal integrity on MRI (FA changes)**
  - **Acquired neurocognitive decline as measured on computer based testing**
    - **Corresponds to WMH burden**
- **Quantitative MRI highly reproducible**



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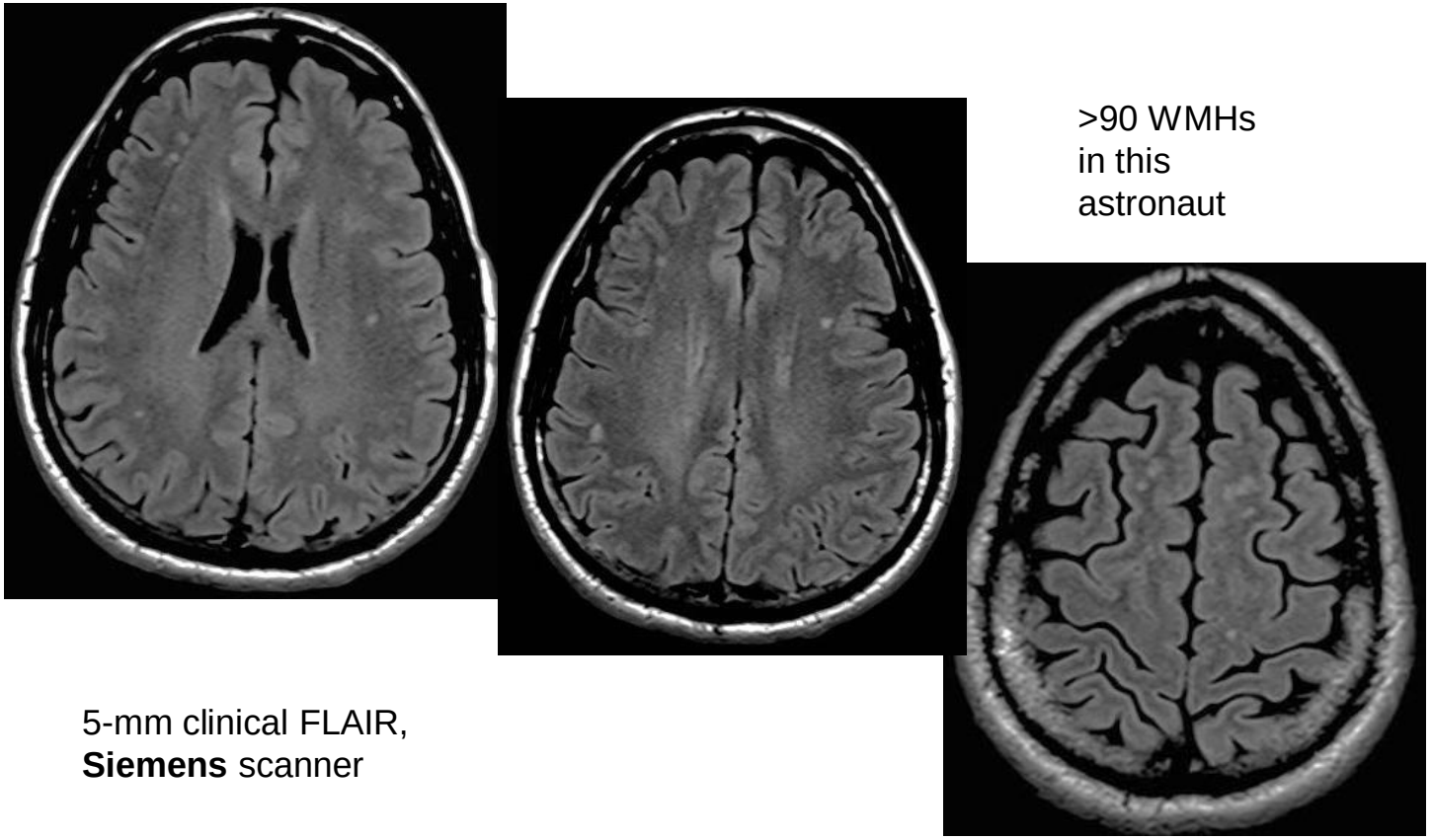
Initial NASA Collaboration

- **Brain MRI scans from 39 astronauts**
  - **41 total scans**
  - **Post ISS or shuttle mission completion**
- **These scans were conducted on 3 different 3T magnets, 2 Siemens scanners, and 1 Philips scanner, with 12-channel head coils**
  - **Siemens n=21; Philips n=20**
- **De-identified MRI scans, 5-mm clinical FLAIR sequence only, were provided by NASA's Lifetime Surveillance of Astronaut Health (LSAH)**
  - **Clinical sequence, not high resolution 3D sequence – underestimates WMH**

MR Imaging



>90 WMHs in this astronaut

5-mm clinical FLAIR, **Siemens** scanner

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Astronaut FLAIR Data

Item	ASTR (mean ± SE)	U2P (mean ± SE)	CTRL (mean ± SE)
Subject number	41	106	320
Age	Withheld	Withheld	28.4 ± 0.5
Total Volume (mL)	0.6618 ± 0.1289	0.8663 ± 0.0502	0.2353 ± 0.100
Total Count	8.61 ± 2.26	12.47 ± 1.45	5.33 ± 0.22
Subcortical Volume (mL)	0.2962 ± 0.1112	0.1295 ± 0.0252	0.0358 ± 0.0037
Subcortical Count	6.51 ± 2.28	7.50 ± 1.38	2.62 ± 0.21
Periependymal Vol	0.3656 ± 0.0327	0.6986 ± 0.0445	0.1995 ± 0.0087
Periependymal Count	2.10 ± 0.07	3.81 ± 0.13	2.72 ± 0.07

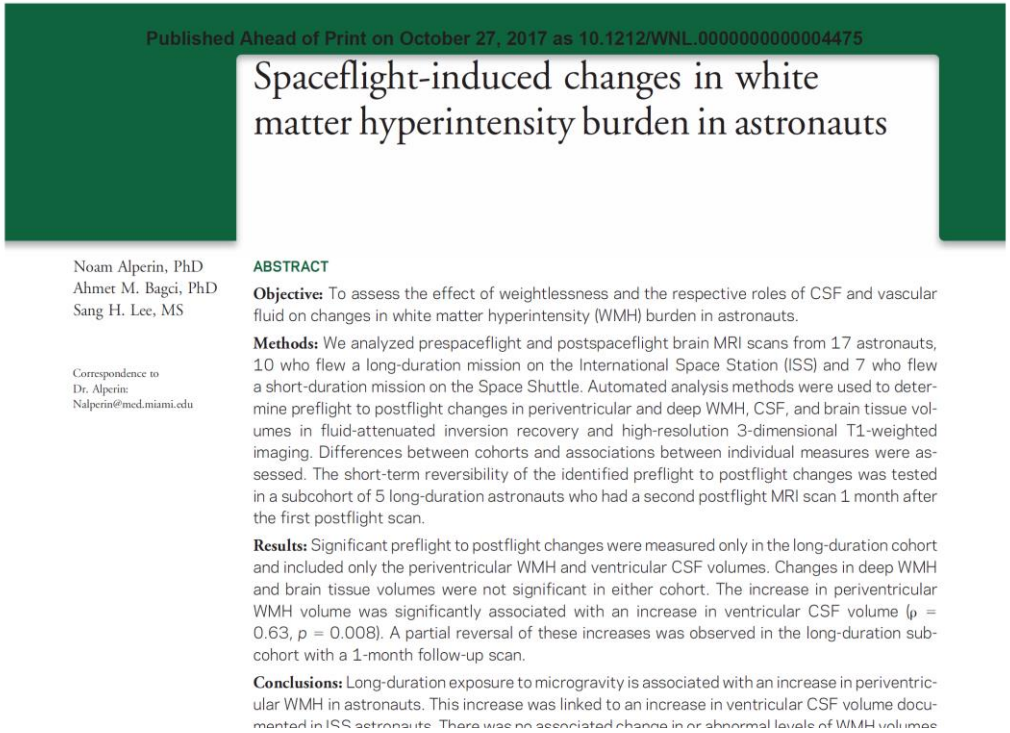
SE = standard error

- Baseline FLAIR MRIs were analyzed for 41 ASTR participants
- Pre- and post-space flight MRIs were available for 34 ASTR
- Pre- and post-EVA MRI scans available in 8 ASTR with open space EVA
- FLAIR MRI was analyzed in 106 U2P (age/gender withheld to preserve nonidentification)
- 320 CTRL recruited from USAF with no occupational exposure to decompression stress
- All USAF participants were healthy and fulfilled Flying Class II/III flight standards as previously described

Astronaut Summary

- **Unexplained increased WMH burden, similar to U-2 pilots**
  - **Is WMH burden in astronauts a consequence of training vs. other factors prior to entry into astronaut corps?**
  - **Exposure to hyper- and hypobaric stresses during training regimen (i.e., chamber activities including underwater/tank training)**
  - **Many exposed to prior activities including aviation (military and commercial), SCUBA diving, mountain climbing, etc.**
- **We don't have the data to be able to draw specific conclusions**
- **Recent demonstration of intracranial fluid shifts, increase in periventricular WMH, and sulcus change with reports of "mental fog" suggests a more detailed analysis of white matter integrity is warranted to understand and minimize risks in these high-performing individuals**

Recent publications



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Spaceflight-induced changes in white matter hyperintensity burden in astronauts

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ABSTRACT

Objective: To assess the effect of weightlessness and the respective roles of CSF and vascular fluid on changes in white matter hyperintensity (WMH) burden in astronauts.

Methods: We analyzed prespaceflight and postspaceflight brain MRI scans from 17 astronauts, 10 who flew a long-duration mission on the International Space Station (ISS) and 7 who flew a short-duration mission on the Space Shuttle. Automated analysis methods were used to determine preflight to postflight changes in periventricular and deep WMH, CSF, and brain tissue volumes in fluid-attenuated inversion recovery and high-resolution 3-dimensional T1-weighted imaging. Differences between cohorts and associations between individual measures were assessed. The short-term reversibility of the identified preflight to postflight changes was tested in a subcohort of 5 long-duration astronauts who had a second postflight MRI scan 1 month after the first postflight scan.

Results: Significant preflight to postflight changes were measured only in the long-duration cohort and included only the periventricular WMH and ventricular CSF volumes. Changes in deep WMH and brain tissue volumes were not significant in either cohort. The increase in periventricular WMH volume was significantly associated with an increase in ventricular CSF volume (ρ = 0.63, p = 0.008). A partial reversal of these increases was observed in the long-duration subcohort with a 1-month follow-up scan.

Conclusions: Long-duration exposure to microgravity is associated with an increase in periventricular WMH in astronauts. This increase was linked to an increase in ventricular CSF volume documented in ISS astronauts. There was no associated change in or abnormal levels of WMH volumes

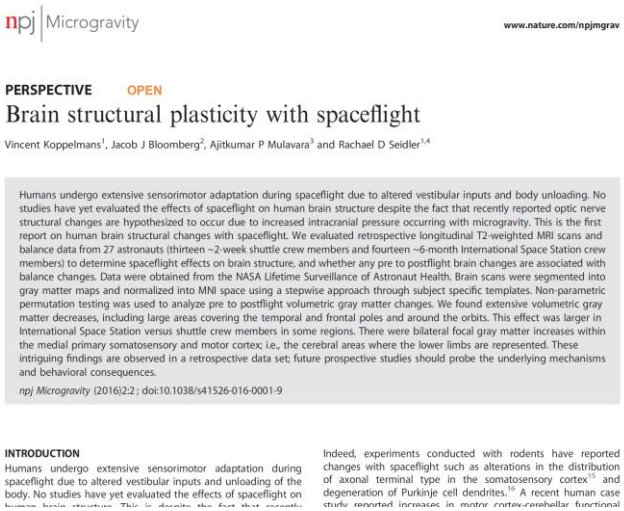
n=17; pre- and post ISS

Alperin, 2017

Concluded increased periventricular WMH but no change in subcortical WMH

Not consistent with our findings

Recent NASA Publications



npj | Microgravity

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PERSPECTIVE OPEN

Brain structural plasticity with spaceflight

Vincent Koppelmans[1], Jacob J Bloomberg[2], Ajitkumar P Mulavara[3] and Rachael D Seidler[1,4]

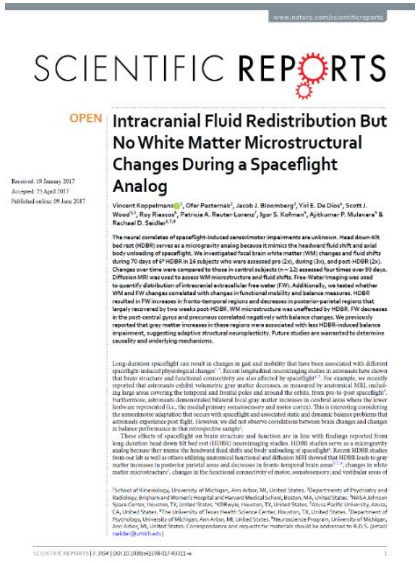
Humans undergo extensive sensorimotor adaptation during spaceflight due to altered vestibular inputs and body unloading. No studies have yet evaluated the effects of spaceflight on human brain structure despite the fact that recently reported optic nerve structural changes are hypothesized to occur due to increased intracranial pressure occurring with microgravity. This is the first report on human brain structural changes with spaceflight. We evaluated retrospective longitudinal T2-weighted MRI scans and balance data from 27 astronauts (thirteen ~2-week shuttle crew members and fourteen ~6-month International Space Station crew members) to determine spaceflight effects on brain structure, and whether any pre to postflight brain changes are associated with balance changes. Data were obtained from the NASA Lifetime Surveillance of Astronaut Health. Brain scans were segmented into gray matter maps and normalized into MNI space using a stepwise approach through subject specific templates. Non-parametric permutation testing was used to analyze pre to postflight volumetric gray matter changes. We found extensive volumetric gray matter decreases, including large areas covering the temporal and frontal poles and around the orbits. This effect was larger in International Space Station versus shuttle crew members in some regions. There were bilateral focal gray matter increases within the medial primary somatosensory and motor cortex; i.e., the cerebral areas where the lower limbs are represented. These intriguing findings are observed in a retrospective data set; future prospective studies should probe the underlying mechanisms and behavioral consequences.

npj Microgravity (2016)2:2 ; doi:10.1038/s41526-016-0001-9

INTRODUCTION

Humans undergo extensive sensorimotor adaptation during spaceflight due to altered vestibular inputs and unloading of the body. No studies have yet evaluated the effects of spaceflight on

Indeed, experiments conducted with rodents have reported changes with spaceflight such as alterations in the distribution of axonal terminal type in the somatosensory cortex[15] and degeneration of Purkinje cell dendrites.[16] A recent human case



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SCIENTIFIC REPORTS

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Intracranial Fluid Redistribution But No White Matter Microstructural Changes During a Spaceflight Analog

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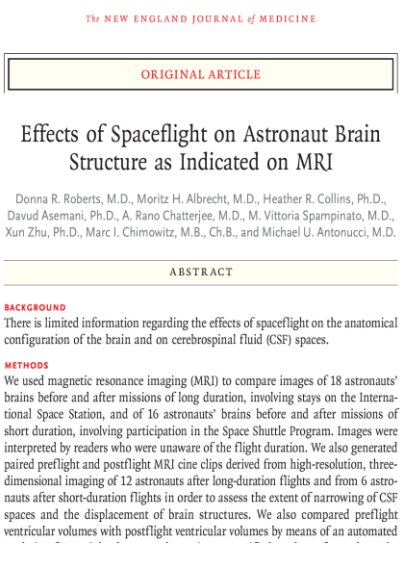
Vincent Koppelmans[1], Ofer Pasternak[2], Jacob J. Bloomberg[3], Yiri E. De Dios[4], Scott J. Wood[3,5], Roy Riascos[6], Patricia A. Reuter-Lorenz[7], Igor S. Kofman[4], Ajitkumar P. Mulavara[4] & Rachael D. Seidler[1,8]

The neural correlates of spaceflight-induced sensorimotor impairments are unknown. Head-down-tilt bed rest (HDBR) serves as a microgravity analog because it mimics the headward fluid shift and axial body unloading of spaceflight. We investigated focal brain white matter (WM) changes and fluid shifts during 70 days of 6° HDBR in 16 subjects who were assessed pre (2x), during (3x), and post-HDBR (2x). Changes over time were compared to those in control subjects (n = 12) assessed four times over 90 days. Diffusion MRI was used to assess WM microstructure and fluid shifts. Free-Water imaging was used to quantify distribution of intracranial extracellular free water (FW). Additionally, we tested whether WM and FW changes correlated with changes in functional mobility and balance measures. HDBR resulted in FW increases in fronto-temporal regions and decreases in posterior-parietal regions that, largely recovered by two weeks post-HDBR. WM microstructure was unaffected by HDBR. FW decreases in the post-central gyrus and precuneus correlated negatively with balance changes. We previously reported that gray matter increases in these regions were associated with less HDBR-induced balance impairment, suggesting adaptive structural neuroplasticity. Future studies are warranted to determine causality and underlying mechanisms.

Long-duration spaceflight can result in changes in gait and mobility that have been associated with different spaceflight-induced physiological changes[1,2]. Recent longitudinal neuroimaging studies in astronauts have shown that brain structure and functional connectivity are also affected by spaceflight[3–5]. For example, we recently reported that astronauts exhibit volumetric gray matter decreases, as measured by anatomical MRI, including large areas covering the temporal and frontal poles and around the orbits, from pre- to post-spaceflight[3]. Furthermore, astronauts demonstrated bilateral focal gray matter increases in cerebral areas where the lower limbs are represented (i.e., the medial primary somatosensory and motor cortex). This is interesting considering the sensorimotor adaptation that occurs with spaceflight and associated static and dynamic balance problems that astronauts experience post flight. However, we did not observe correlations between brain changes and changes in balance performance in that retrospective sample[3].

These effects of spaceflight on brain structure and function are in line with findings reported from long-duration head-down tilt bed rest (HDBR) neuroimaging studies. HDBR studies serve as a microgravity analog because they mimic the headward fluid shifts and body unloading of spaceflight[6]. Recent HDBR studies from our lab as well as others utilizing anatomical functional and diffusion MRI showed that HDBR leads to gray matter increases in posterior parietal areas and decreases in fronto-temporal brain areas[7–9]; changes in white matter microstructure[7]; changes in the functional connectivity of motor, somatosensory, and vestibular areas of



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Effects of Spaceflight on Astronaut Brain Structure as Indicated on MRI

Donna R. Roberts, M.D., Moritz H. Albrecht, M.D., Heather R. Collins, Ph.D., Davud Asemani, Ph.D., A. Rano Chatterjee, M.D., M. Vittoria Spampinato, M.D., Xun Zhu, Ph.D., Marc I. Chimowitz, M.B., Ch.B., and Michael U. Antonucci, M.D.

ABSTRACT

BACKGROUND

There is limited information regarding the effects of spaceflight on the anatomical configuration of the brain and on cerebrospinal fluid (CSF) spaces.

METHODS

We used magnetic resonance imaging (MRI) to compare images of 18 astronauts' brains before and after missions of long duration, involving stays on the International Space Station, and of 16 astronauts' brains before and after missions of short duration, involving participation in the Space Shuttle Program. Images were interpreted by readers who were unaware of the flight duration. We also generated paired preflight and postflight MRI cine clips derived from high-resolution, three-dimensional imaging of 12 astronauts after long-duration flights and from 6 astronauts after short-duration flights in order to assess the extent of narrowing of CSF spaces and the displacement of brain structures. We also compared preflight ventricular volumes with postflight ventricular volumes by means of an automated

From the Department of Radiology and Radiological Science, Division of Neuroradiology (D.R.R., M.H.A., H.R.C., D.A., A.R.C., M.V.S., M.U.A.), and the Department of Neurology (M.I.C.), Medical University of South Carolina, Charleston; the Department of Diagnostic and Interventional Radiology, University Hospital Frankfurt, Frankfurt, Germany (M.H.A.); and the Department of Psychology, Normal College, Shihezi University, Xinjiang, China (X.Z.). Address reprint requests to Dr. Roberts at the Department of Radiology and Radiological Science, 6 Jonathan Lucas St., MSC 323, Medical University of South Carolina, Charleston, SC 29425-3230, or at robertdr@musc.edu.

Koppelmans/Seidler – 2016 and 2017 respectively; Roberts 2017

Cleared, SAF/PA, Case # 2018-0546, 25 Sep 2018.

Recent Publications

Brain Tissue–Volume Changes in Cosmonauts

TO THE EDITOR:

Long-duration spaceflight has detrimental effects in several physiological systems. Several studies have shown an upward shift of the cerebral hemispheres, a decrease in frontotemporal volume, and an increase in ventricle size after spaceflight.[1-3] However, information is limited about the effects of microgravity on brain volume, particularly regarding changes that are evident more than 1 month after spaceflight.

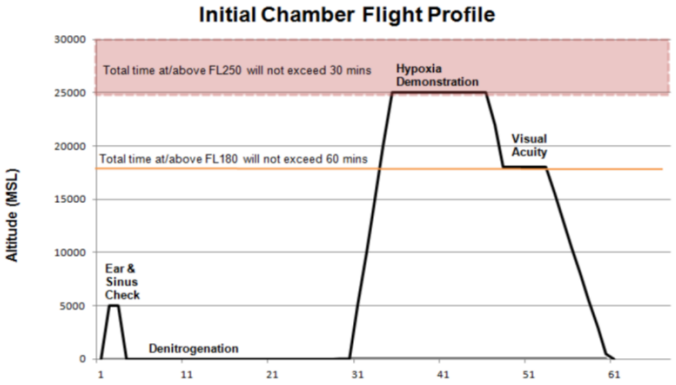
October 25, 2018
N Engl J Med 2018; 379:1678-1680
DOI: 10.1056/NEJMc1809011
Metrics

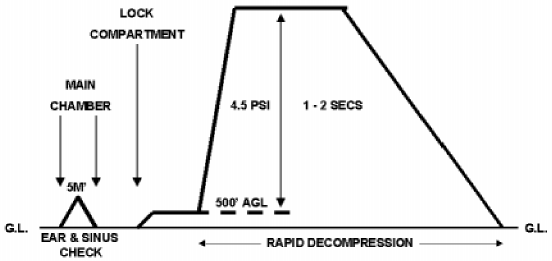
NEJM CareerCenter

Ombergen et al. 2018

Single Hypobaric Exposure Study

- **Hypothesis – single occupational exposure to hypobaria and/or hypoxia will be associated with transient MRI and serological changes**
- **Identifying transient changes with single exposure may lead to understanding the neuropathophysiology of white matter injury demonstrated in chronic hypobaric exposure**
- **Only volunteers undergoing occupational training hypobaric and/or hypoxic exposures (direction by USAF/SG); 25,000 ft; (7,620 m, 5.45 psi)**





Single Exposure Study

- Examine acute (MRI/serological) changes following a single exposure – all meet FCII/FCIII neurological standards
  - 1. Hypobaric-hypoxic (AFC – aircrew chamber training)
  - 2. Hypobaric (AOP inside safety monitors)
  - 3. Hypoxic (ROBD – reduced O_2 breathing device)
  - 4. NOR – controls

- Protocol:
  - MRI 24 h before; 24 h after; 72 h after
  - Serological immediately before; immediately after; 24 h after; 72 h after
  - *No other altitudinal exposure beginning 7 d prior
  - *No alcohol beginning 7 d prior
  - Maintain normal physiological activities
    - No sleep deprivation/shift changes, etc.
- Intra-subject and cross-group comparisons

Single Exposure Study

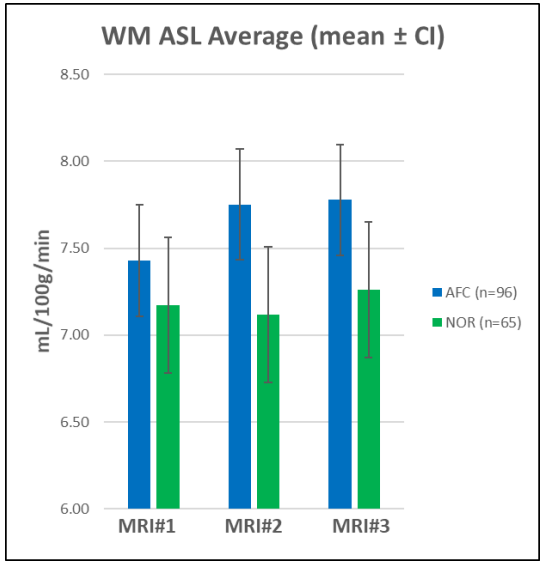
- Total of 178 total subjects
- AFC group – 96 (32F, 64M)
  - Avg. age 21.2
- NOR – 65 (6F, 59M)
  - Avg. age 22.4
- AOP – 14
- ROBD – 3

Siemens 3T Verio

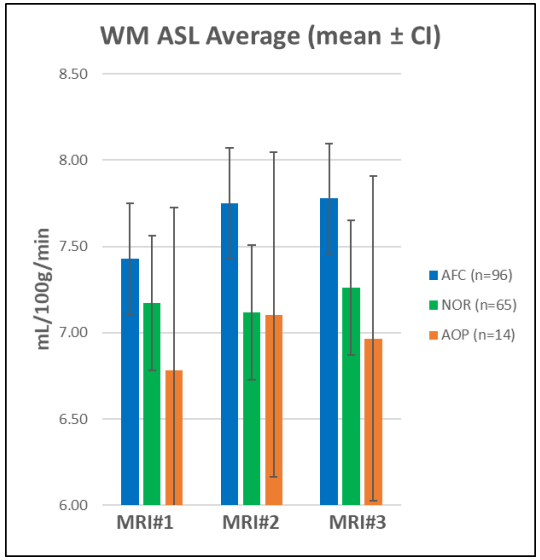
- Recruitment challenges for AOP and ROBD groups
  - Parameters meant “no flying” duties for almost 2 weeks as a volunteer, which was unrealistic
  - Follow on study using Brooks City Base subject panel is planned for 2020 to address the “hypoxia effect”

Arterial Blood Flow (ASL) – CBF

- **Increase in WM CBF at 24/72 h**
  - **Significant group (AFC vs. NOR) difference**
    - **WM p<0.001 (Utilized generalized additive model adjusted for age and gender)**
- **Potentially similar change in AOP group ("n" too small for assessment)**



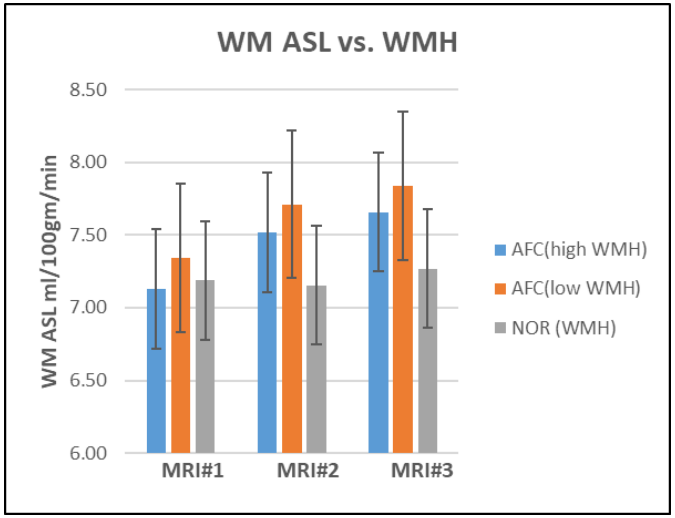
AFC	Subj #	WM
MRI#1 avg	*96*	*7.43*
MRI#2 avg	*94*	*7.75*
MRI#3 avg	*96*	*7.78*
TTEST #1-#2		*0.004*
TTEST #1-#3		*0.009*
TTEST #2-#3		*0.967*
NOR		
MRI#1 avg	*65*	*7.17*
MRI#2 avg	*65*	*7.12*
MRI#3 avg	*60*	*7.26*
TTEST #1-#2		*0.738*
TTEST #1-#3		*0.363*
TTEST #2-#3		*0.088*



Cleared, SAF/PA, Case # 2018-0546, 25 Sep 2018.

Single Exposure
MR FLAIR and FA Average

- Cerebral blood flow appears to be associated with the preexisting FLAIR WMH burden
- Higher WMH baseline associated with greater WM-ASL response to stress



Cerebral Blood Flow

- No change in normal controls, as expected
- Approximately 6% increase in WM CBF
- Increase CBF reflects increased cerebral demand
  - Inflammatory, metabolic, ischemic
- Does exposure induce transient WM damage?
  - Need for adequate recovery time between exposures?
  - Underlying physiological explanation remains unclear

Phase 2 Single Exposure MR Spectroscopy

- **Reproducible measurement of multiple neurometabolites with MR spectroscopy (TE30) in frontal (white matter) and anterior cingulate gyrus (mixture of white and gray matter)**
  - **Glu=glutamate**
  - **tCho=choline**
  - **tNAA=n-acetylasparate**
  - **mI=myo-inositol**
  - **tCr=creatine**
  - **Glu+Gln=glutamate + glutamine**
  - **GSH=glutathione**
- **tNAA reflects neurons**
- **mI reflects glia**
- **GSH reflects oxidative stress**
- **tCr reflects energy**

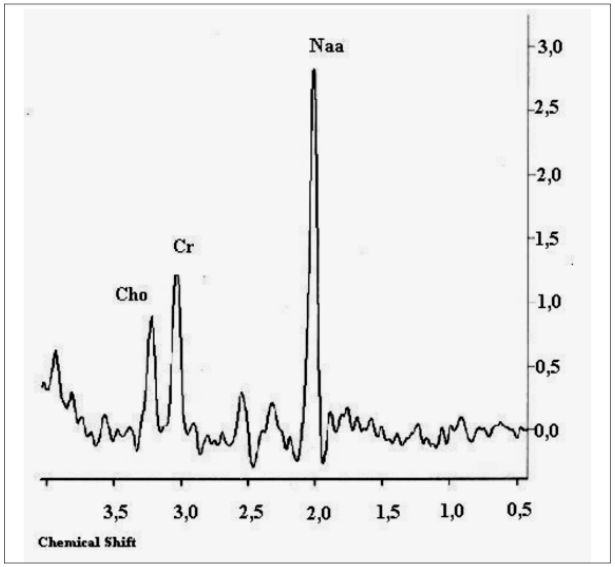


Figure 1. Normal brain curve from proton magnetic resonance spectroscopy of the brain, showing peaks of the metabolites N-acetyl aspartate (Naa), creatine (Cr) and choline (Cho), with echo time of 136 milliseconds.

McGuire et al. Brain Behav 2017;e00759 (https://doi.org/10.1002/brb3.759)

Single Exposure
MR Spectroscopy

- **Significant group differences**
  - Generalized additive model statistics
  - NAA=neuronal
  - mI=glial
  - Cr=creatine
  - Glu+Gln=glutamate + glutamine
  - GSH=oxidative stress
- **Significant differences for:**
  - GSH Front 30 (p=0.029)
  - Glu Front 30 (p=0.017)
  - Cho AC 30 (p=0.009)
  - NAA AC 30 (p=0.023)
  - MI AC 30 (p=0.038)
  - Cr AC 30 (p=0.008)
  - GluGln AC 30 (p=0.004)
- **Metabolites return to normal on MRI #3**

TE30 Frontal Average	*Count*	*Average Glu*	*Average Cho*	*Average NAA*	*Average mI*	*Average Cr*	*Average Glu+Gln*	*Average GSH*
AFC#1	*89*	*8.177*	*2.253*	*10.136*	*5.362*	*7.152*	*9.831*	*2.444*
AFC#2	*87*	*8.093*	*2.229*	*10.000*	*5.265*	*7.051*	*9.809*	*2.381*
AFC#3	*89*	*8.120*	*2.260*	*10.118*	*5.297*	*7.178*	*9.929*	*2.403*
AFC Paired TTEST p-value								
#1-#2		*0.435*	*0.175*	*0.110*	*0.047*	*0.130*	*0.944*	*0.170*
#1-#3		*0.481*	*0.681*	*0.826*	*0.430*	*0.654*	*0.291*	*0.523*
#2-#3		*0.884*	*0.202*	*0.292*	*0.582*	*0.091*	*0.414*	*0.604*
NOR#1	*60*	*8.356*	*2.259*	*10.170*	*5.368*	*7.251*	*10.194*	*2.470*
NOR#2	*59*	*8.206*	*2.273*	*10.195*	*5.419*	*7.191*	*10.093*	*2.471*
NOR#3	*54*	*8.259*	*2.268*	*10.095*	*5.361*	*7.184*	*10.085*	*2.460*
NOR Paired TTEST p-value								
#1-#2		*0.141*	*0.683*	*0.855*	*0.612*	*0.409*	*0.407*	*0.870*
#1-#3		*0.445*	*0.879*	*0.428*	*0.899*	*0.491*	*0.413*	*0.795*
#2-#3		*0.364*	*0.884*	*0.861*	*0.646*	*0.949*	*0.429*	*0.461*

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Phase 2 Single Exposure MR Spectroscopy

- **Cerebral blood flow increase correlates with cellular metabolite changes**

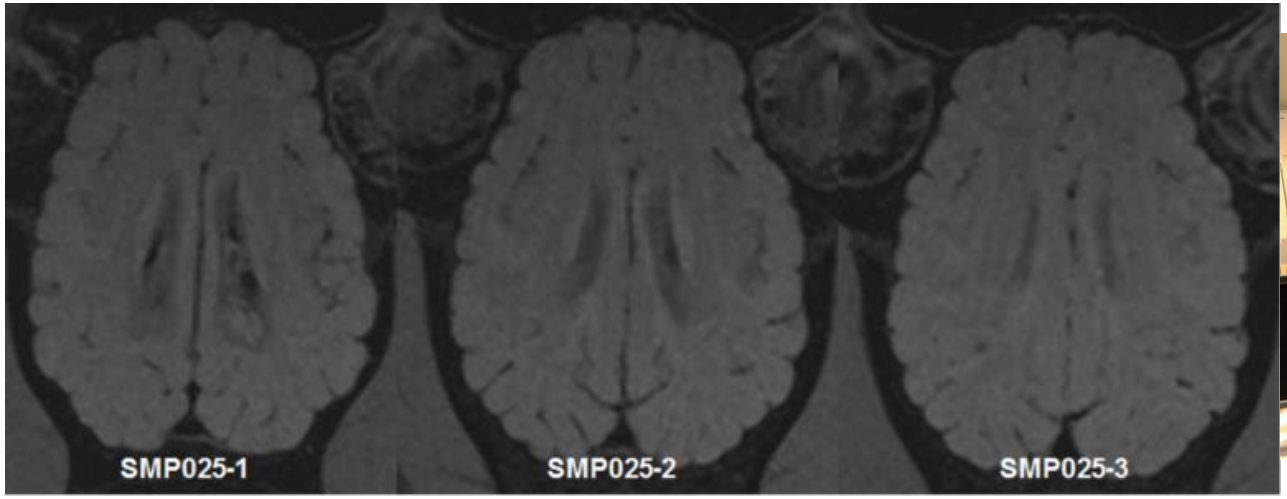
Metabolite	WM-ASL
TE30 Frontal Lobe WM	**p-value**
Mean Glu	0.061
Mean tCho	0.013
Mean tNAA	0.589
Mean mI	0.001
Mean tCr	0.001
Mean Glu+Gln	0.148
Mean GSH	0.122
TE30 Ant Cingulage GM	
Glu	0.05
GSH	0.011
tCho	0.611
tNAA	0.045
mI	0.641
tCr	0.37
Glu+Gln	0.018

Single Exposure Summary

- **Single occupational exposure to a hypobaric/hypoxic environment is associated with an increase in CBF**
  - **CBF tightly regulated by cerebral metabolic demands**
  - **Hypoxic portion ~ 2-5 min (correlating with a PaO_2Sat ~ 65-75%)**
- **The degree of ASL/CBF change appears related to baseline neurocellular metabolites**
- **The greater the initial WMH burden the greater the ASL response**
  - **Is there an inherent predisposition for injury?**
- **Duration of CBF changes – 5 MRI study (MRI#4 and #5 at 5 and 7 days post exposure; CBF normalizes on MRI#4 – study will be completed in 2019)**

Swine Studies

- **Develop an animal model for axonal cerebral injury following high-altitude (non-hypoxic hypobaric) exposure utilizing advanced magnetic resonance imaging techniques**

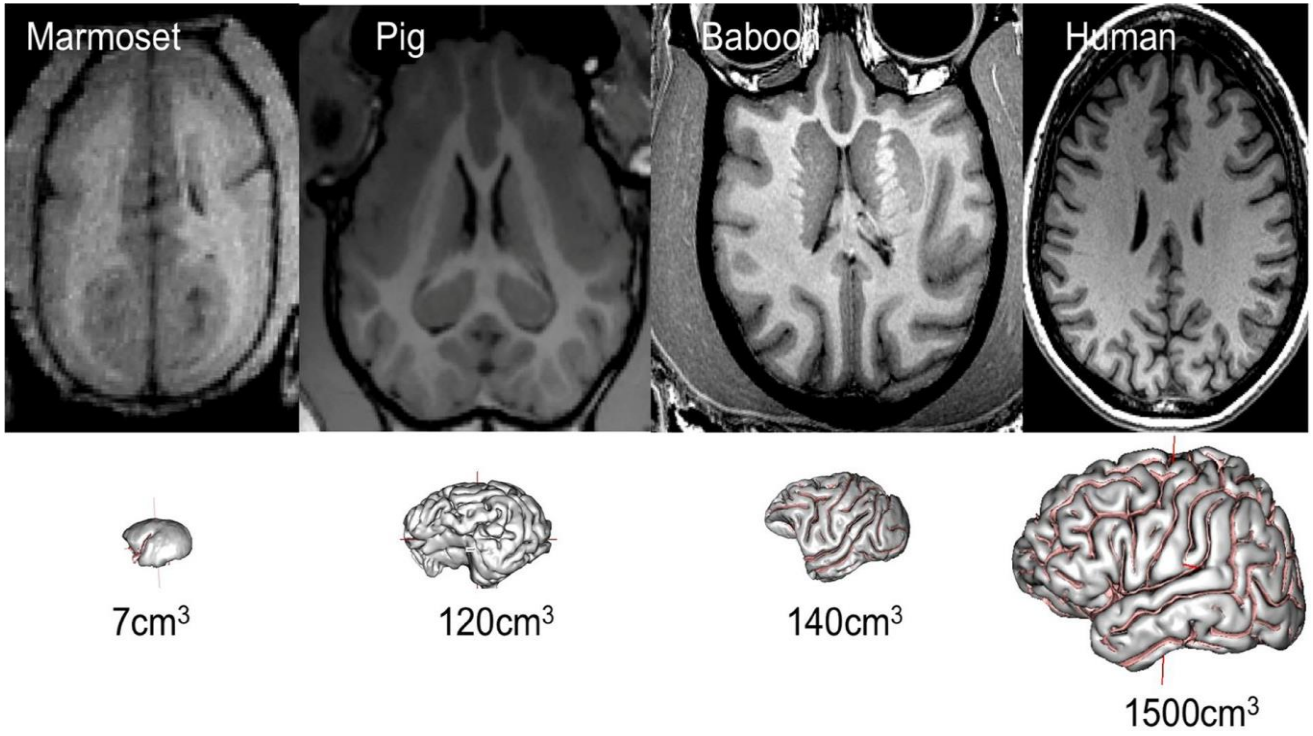


Siemens 3T Verio

Why Swine?

- *Gyrencephalic model for brain development*
- *Similar brain myelination and white matter development pattern to humans*
- *Believe it is a translational model*
- *Alternative to rodents (lissencephalic brain)*
- *Alternative to non-human primates (more ethical constraint; higher cost)*
- *Use of "adolescent" minipigs*
  - *Ages: Average at MRI#1 102.58 days +- 16.91 days*
    - *Average at MRI#2 123.58 +- 17.1 days; avg MRI #3 149.5 +- 15.96 days*
  - *Corresponds to humans approximately ages 10-19*
  - *Females – bladder catheterization purposes*
  - *Human knee coil for MRI – thus requirement for minipigs*
  - *Larger animals -> no current MRI coil with satisfactory SNR*
- *MRI protocol near equivalent to human MRI high-altitude study*
  - *3 hour acquisition time for swine; about 2 hours for humans*

Brains of different species



Axial slices and 3D volume rendering for brains of different species

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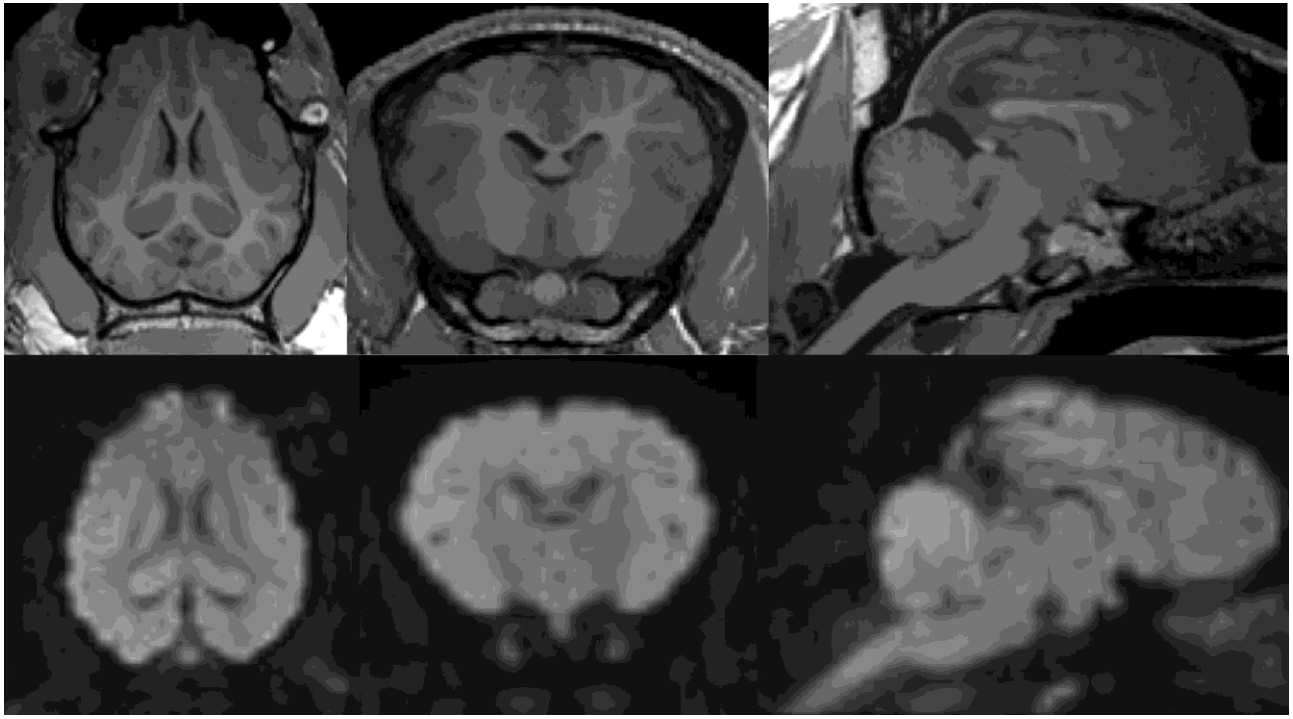
Sus Scrofa domestica Brain MRI



T1-weighted and DWI (avg across all b-values) images. Fully gyrified cortex with excellent gray-white matter differentiation. DWI demonstrates excellent resolution and lack of shape distortion artifact.

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Current Swine Model

Mimic U2 pilot experience

- **No sedation**
- **1 h 100% O_2 pre-breathe**
- **30 min ascent**
- **8 h at altitude**

	POD 1&2	POD 3&4	POD 5&6	POD 7&8	POD 9&10	POD 11
n	12	12	12	16	16	8
altitude	30,000 ft	5,000 ft	785 ft	30,000 ft	30,000 ft	5,000 ft
air	95%+ O2	room air	95%+ O2	95%+ O2	95%+ O2	room air
flights	6	6	6	12	12	12
frequency	q3d	q3d	q3d	q2d	q2d	q2d

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Current Swine Model

MRIs obtained

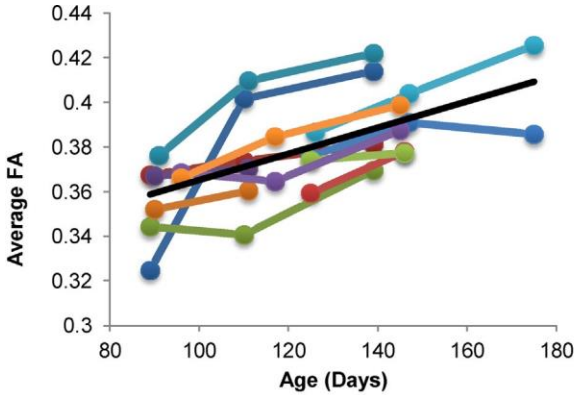
- **Prior to exposure**
- **5-6h post exposure**
- **4 weeks post final exposure**
- **8 weeks post final exposure (PODs 7-11)**

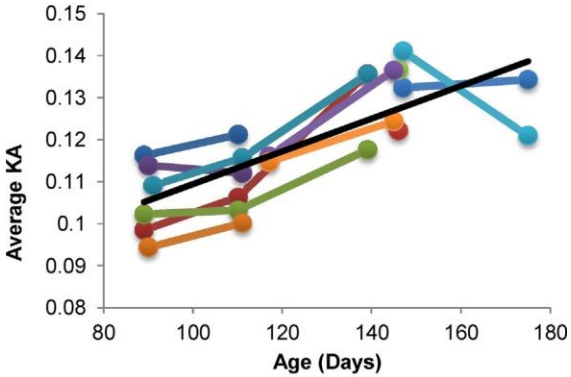
By the 4 week post-exposure MRI session, the POD 1&2 pigs were already growing too big for the head coils.
Younger animals(~8 weeks) were used for subsequent pods.

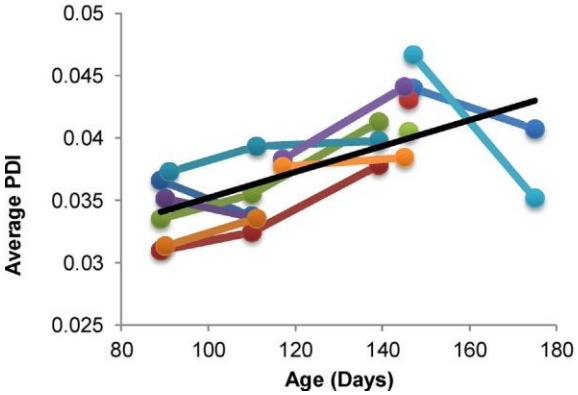
Current Swine Study

- ***Final group completed last MRI 4 Nov 18***
- ***June 2018 – agreement with the Center for Neuroscience and Regenerative Medicine (CNRM) to evaluate our neuropathology data***
  - ***Lack of DoD veterinary neuoropathology expertise in San Antonio, TX***
- ***Only preliminary exposure group data at this time***
- ***Follow on study in 2019 will look at the effects of anti-inflammatory medications administered pre-flight using MRI, neuroinflammatory markers, and neuropathology***

Fractional Anisotropy (FA), Kurtosis Anisotropy (KA) and Permeability-Diffusion Index (PDI)







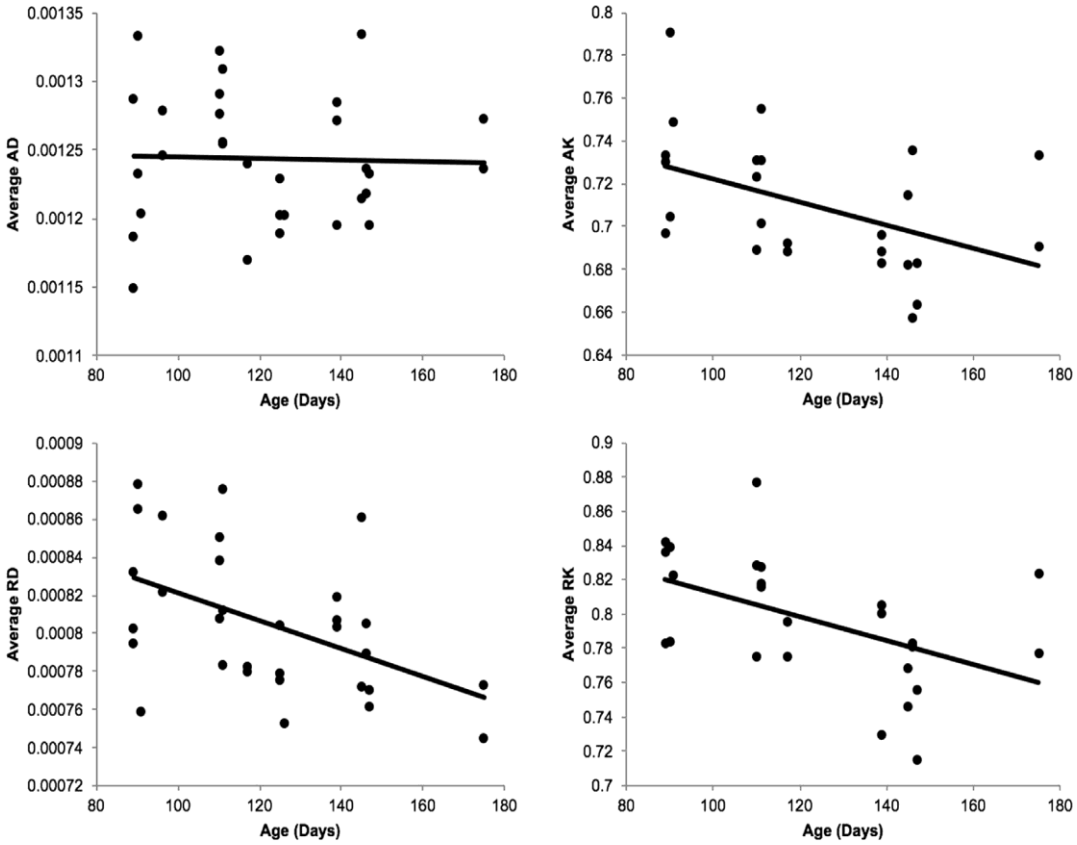
- Normal controls

- FA, KA and PDI showing significant longitudinal effects of age

- Black line is the fitted regression line

- Average whole brain diffusion trajectory for each individual pig in color

AD, RD, AK, RK Diffusion Measures

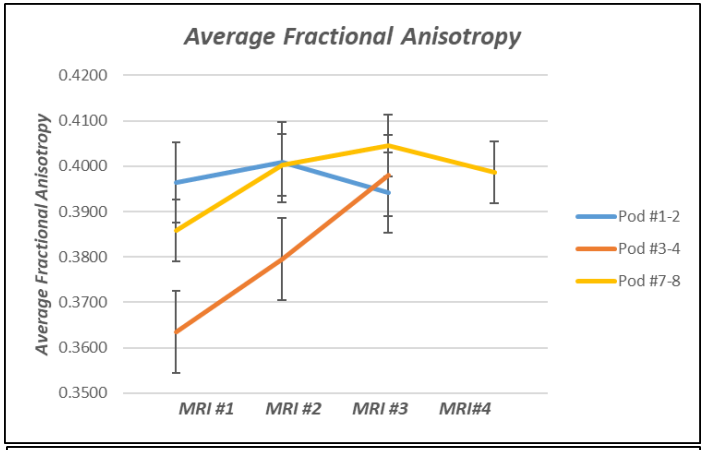


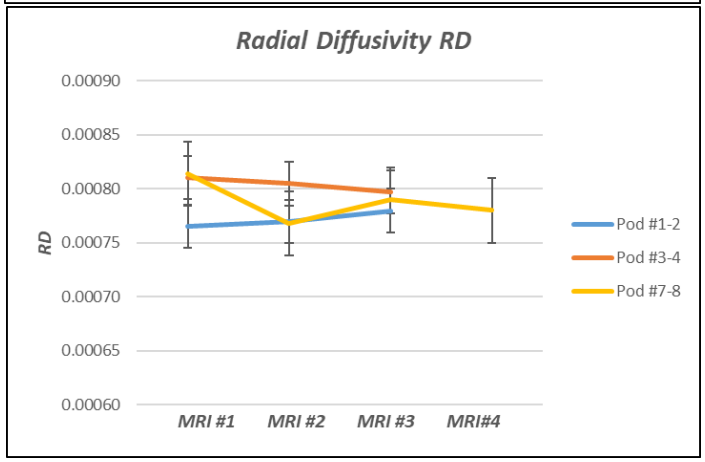
AD – axial diffusivity
RD – radial diffusivity
AK – axial kurtosis
RK - radial kurtosis

Significant changes with age

Ryan CR, Sherman PM, et al. J Neuroscience Methods 296 (2018): 99-108.

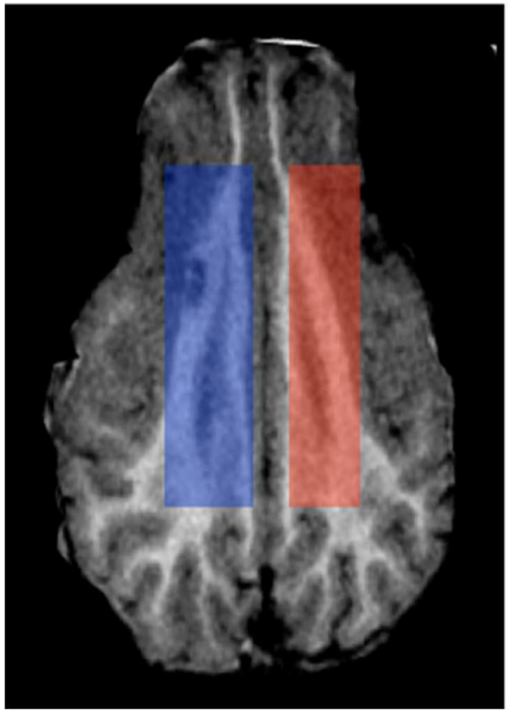
DWI Post Hypobaric Exposure

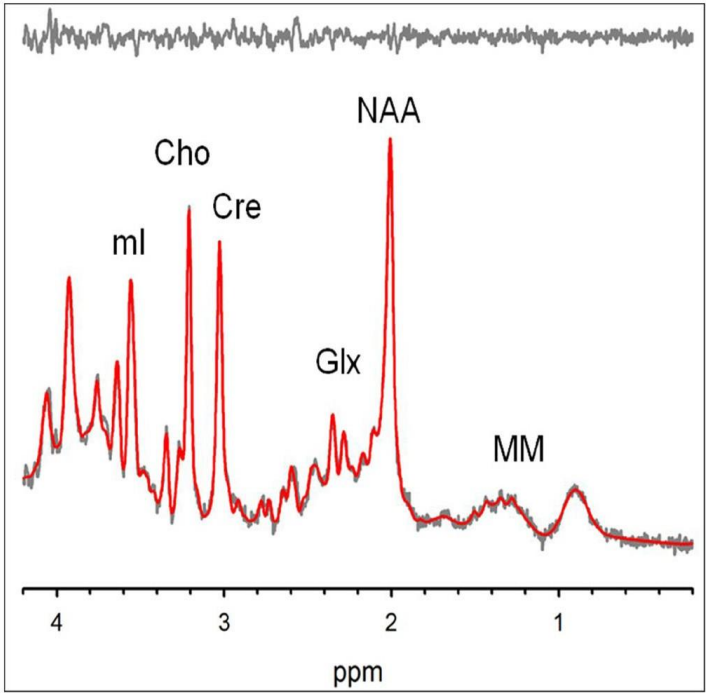




- **Pod 1&2, six exposures over 18 days**
  - **Older animals**
- **Pod#7-8, twelve exposures over 24 days**
  - **Similar age to Pod 3&4 controls**
- **FA drops while RD increases**
  - **Suggests vasogenic (interstitial) edema**

Brain Magnetic Resonance Spectroscopy Model



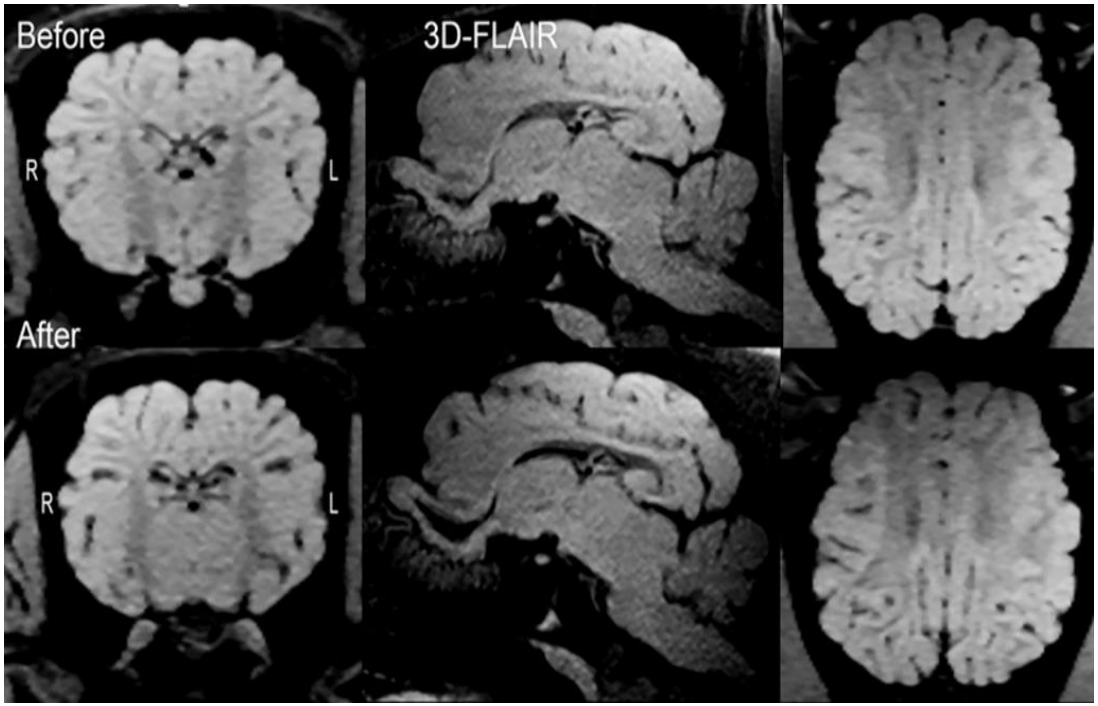


MRS findings mimic human adolescent development in controls

MRS findings mimic human changes in exposed subjects

Representative place of 2 spectroscopic voxels in the pig's brain; representative spectrum of metabolite peaks identified as offset (parts per million from hydrogen frequency)

Swine FLAIR Imaging



Pre and post-exposure FLAIR imaging in 3-planes.
No white matter hyperintensities with extreme altitude exposure as seen in humans.

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Summary

- ***Recurrent exposure to nonhypoxic extreme hypobaria incites:***
  - ***Focal punctate subcortical white matter hyperintensities (WMH) on MRI***
  - ***Diffuse decrement in axonal integrity on MRI***
  - ***Acquired neurocognitive decline as measured on CBT***
  - ***Clinical neurological decompression sickness is not a prerequisite for abnormalities***
- ***Single exposure to extreme hypobaria/hypoxia (routine occupational aircrew training) incites:***
  - ***Increase in white matter cerebral blood flow, persists at 72 hrs post-exposure on MRI; normal 5 days post exposure***

- ***Quantitative serial MRI highly reproducible – in humans and swine***
- ***Swine model may be a viable model***

McGuire et al. Neurol 2013;81:729-735
McGuire et al. Ann Neurol 2014;76:719-726
McGuire et al. Neurol 2014;83:638-645
McGuire et al. Aerosp Med Hum Perform 2016;87:983-988
McGuire et al. Brain Behav 2017;e00759 (https://doi.org/10.1002/brb3.759)

Summary - Unknowns

- **Pathophysiological mechanism(s)**
  - Relative contribution of hypobaria vs. other metabolic parameters (hyper-/hypoxemia, hyper-/hypocarbia, etc.)
  - Temporal susceptibility window? Repeated exposure without recovery; "Double-hit hypothesis"
- **Individual biosusceptibility**
  - Intial genomic APOE4 study in U2 and AOP subjects was negative
- **Possible mitigating/treatment strategies**
  - Swine anti-inflammatory study in 2019
- **Possible impact on acutely injured brain**
  - Ongoing animal research
- **Long-term impact on neurocognition**
  - Recently imaged 2 former U2 and SR71 pilots (90 yo is still flying his private aircraft); MRI analysis pending

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QUESTIONS?