Subcortical Brain Injury: Relationship to Hypobaric Exposure
Johnson Space Lab
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- Opinions are those of the authors
U-2 Environment

- **U-2 operates in extreme environment**
- **Crew protection based on years of experience and research**
Neurologic Decompression Sickness (NDCS) Background

- Increased incidence of NDCS associated with SWA conflict
  - 1955-1998 no “reported” type II
  - 1996 anonymous survey 75.5% during career noted DCS
  - 2002-2009 16 confirmed NDCS events (5 near-fatal)

1994-2005
- DCS risk 0.076%/flight
- 0-5 cases/yr
- 10 type II/12 yr

2006-2010
- 300% increased rate of NDCS
- DCS risk 0.23%/flight
- 6-10 cases/yr
- 22 type II/5 yr

Jersey et al. Aviat Space Environ Med 2010; 81:64-8
Jersey et al. Aviat Space Environ Med 2011; 82:673-828
Hundemer et al. Aviat Space Environ Med 2012; 83:968-74
WMH and “embolic” lesions

Initial U2P Pilot Evaluations at ACS
MRI in U2P (U-2 Pilots) With & Without Clinical NDCS
MRI Transformation Process for Quantitative Assessment

- Nonbrain removed from FLAIR image
- FLAIR registered to T1-weighted image
- Registered to Talairach-atlas-based stereotactic frame
- Lesions analyzed using Talairach-based boundaries
Quantitative Analysis of White Matter Hyperintensities (WMH)

- Significantly increased WMH volume & count in PHY and U2P vs. DOC
- Johnckherre-Terpstra test suggests relationship to exposure intensity
  - DOC < PHY ≤ U2P on WMH volume (p=0.024)
  - DOC < PHY ≤ U2P on WMH count (p=0.012)
  - PHY < U2P not significant
- PHY – U2P equivalency on Kolmogorov-Smirnov test (p=0.388)
- Non-normalcy distribution on Shapiro-Wilk test (p < 0.001) → nonparametric statistics

<table>
<thead>
<tr>
<th></th>
<th>DOC (n=162)</th>
<th>PHY (n=83)</th>
<th>U2P (n=106)</th>
<th>Mann-Whitney-Wilcoxon Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±std dev</td>
<td>mean±std dev</td>
<td>mean±std dev</td>
<td>DOC:PHY</td>
</tr>
<tr>
<td>WMH volume (mL)</td>
<td>0.035±0.058</td>
<td>0.126±0.404</td>
<td>0.146±0.294</td>
<td>p=0.029</td>
</tr>
<tr>
<td>WMH count</td>
<td>2.8±3.2</td>
<td>6.4±11.1</td>
<td>9.6±18.2</td>
<td>p=0.050</td>
</tr>
</tbody>
</table>

McGuire et al. Neurol 2013;81:729-735
Relative Distribution of WMH

- PHY and U2P have a diffuse dissemination of WMH
- Frontal dominant in all groups
- Suggests a shower of microemboli c/w dominance of ICA flow
DOC < PHY ≤ U2P all lobes

 Possibly supports a relationship to “intensity” of exposure

 WMH distribution driven predominantly by frontal lobe burden

 Supports hypothesis of relationship to ICA blood flow
Total Exposure vs. WMH

Total hours of exposure weakly correlates with WMH burden

Correlation of WMH Volume and Count with Total Hours High-Altitude Exposure

<table>
<thead>
<tr>
<th>Exposure (hours) versus WMH vol/cnt in PHY-U2P</th>
<th>WMH Volume</th>
<th>WMH Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean / median / std dev / CI</td>
<td>Spearman’s rho</td>
<td>Linear regression r²</td>
</tr>
<tr>
<td>97 / 73 / 88 / 78-116 PHY (n=83)</td>
<td>-0.002</td>
<td>0.008</td>
</tr>
<tr>
<td>741 / 667 / 489 / 648-834 U2P (n=105)</td>
<td>0.144</td>
<td>0.002</td>
</tr>
<tr>
<td>461 / 219 / 491 / 391-531 PHY+U2P (n=188)</td>
<td>0.141</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Clinical NDCS vs. WMH

- Clinical NDCS increases with hours of exposure
- Slight linear relationship of NDCS to WMH burden
- Suggests WMH are occurring both with and without a clinical diagnosis of NDCS
- Implies cannot solely rely on clinical symptoms for a diagnosis of subcortical WMH burden

<table>
<thead>
<tr>
<th></th>
<th>WMH vol $r^2$</th>
<th>WMH cnt $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDCS</td>
<td>0.017</td>
<td>0.003</td>
</tr>
<tr>
<td>No NDCS</td>
<td>0.025</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Age vs. WMH

Small correlation of WMH to age

Correlation of WMH Volume and Count with Age

<table>
<thead>
<tr>
<th>Age (years) versus WMH vol/cnt in DOC-PHY-U2P</th>
<th>WMH Volume</th>
<th>WMH Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean / median / std dev / CI</td>
<td>Spearman’s rho</td>
<td>Linear regression r²</td>
</tr>
<tr>
<td>34.6 / 33.0 / 5.8 / 33.6-35.5 DOC (n=148)</td>
<td>0.189</td>
<td>0.017</td>
</tr>
<tr>
<td>36.5 / 36 / 7.1 / 34.5-37.5 PHY (n=83)</td>
<td>0.084</td>
<td>0.023</td>
</tr>
<tr>
<td>37.7 / 37.5 / 36.6-38.8 U2P (n=105)</td>
<td>0.142</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Relationship of Age to WMH (all U2P – PHY – DOC)

- Subcortical WMH volume/count modestly increase with each 5-year age interval
  - Possibly implies weak relationship to total exposure
- Relative increase in U2P & PHY compared to DOC maintained in each 5-year interval age 26-50
  - Variation in intervals between PHY and U2P unclear

Relationship of Age to WMH (exclude HTN and/or HLD)

- DOC < PHY ≤ U2P in every 5-year interval after excluding HTN and/or HLD
- Generally increase WMH burden per 5-year interval in each group
- Study entry criteria excluded any subject with more than mild HTN and/or HLD
- Suggests mild HTN and/or mild HLD not explanation for findings

No difference between DOC (n=141) and PHY (n=83) on FA values (average or tract) (p > 0.05)

Cannot directly compare DOC & U2P (different MRI scanners)

Data very preliminary
**DTI in U2P**

**High vs. Low WMH Vol/Cnt**

- **No overall or tract difference between low vs. high WMH vol/cnt** $(p > 0.05)$
  - Low/High WMH volume $(n=34/68)$ & count $(n=36/66)$

- **Separation point based on median DOC WMH values**

  **U2P DTI**

  **Low vs. High WMH**

  DOC median separation

![Graph showing DTI values for U2P Low vs. High WMH Vol and Cnt with tracts labeled from 1 to 67.](image)
Overall no correlation between total average FA to total WMH volume (-0.013/0.9) or count (0.01/0.918)

Borderline correlation of multiple tracts to insular lobe c/w density of traversing tracts

Suggests possibility of conduction alteration

<table>
<thead>
<tr>
<th>Tract</th>
<th>Insular volume (Spearman rho/p-value)</th>
<th>Insular count (Spearman rho/p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA Average</td>
<td>-0.182/0.067</td>
<td>-0.171/0.086</td>
</tr>
<tr>
<td>FA Fornix</td>
<td>-0.182/0.067</td>
<td>-0.171/0.087</td>
</tr>
<tr>
<td>FA Corona radiate</td>
<td>-0.175/0.078</td>
<td>-0.165/0.097</td>
</tr>
<tr>
<td>FA Thalamic radiation</td>
<td>-0.195/0.050</td>
<td>-0.182/0.067</td>
</tr>
<tr>
<td>FA Sagital striatum</td>
<td>-0.236/0.017</td>
<td>-0.232/0.019</td>
</tr>
<tr>
<td>FA External capsul</td>
<td>-0.186/0.061</td>
<td>-0.179/0.071</td>
</tr>
<tr>
<td>FA Cingulum</td>
<td>-0.178/0.073</td>
<td>-0.166/0.094</td>
</tr>
<tr>
<td>FA Fronto-occipital</td>
<td>-0.173/0.083</td>
<td>-0.152/0.127</td>
</tr>
</tbody>
</table>
Pending Imaging Analyses

- **Cortical mapping**
  - Utilizing FreeSurfer to map cortical thickness
  - Challenged by cross-scanner comparisons

- **MR spectroscopy comparison**
  - Total NAA ("tNAA") = \( N\)-acetylaspartate (NAA) plus \( N\)-acetylaspartylglutamate (NAAG)
    - Marker of neuronal viability and function
  - Total Cr ("tCr") = creatine plus phosphocreatine
    - Index of energy metabolism
  - Total Choline ("tCho") = glycerophosphochocholine plus phosphocholine
    - Reflective of membrane turnover
MAB-II (current)  
U2P vs. AFP

- No baseline differences between U2P and AFP (USAF pilot controls) at time of UPT (undergraduate pilot training)
- No difference between U2P and AFP on AFOQT
- No difference between U2P and AFP at time of UPT on MAB-II
- Similar distribution of initial platform (attack/fighter, instructor pilot, heavy) assignment out of UPT
- No significant difference in current MAB-II

<table>
<thead>
<tr>
<th>MAB-II</th>
<th>U2P (n=87)</th>
<th>AFP (n=83)</th>
<th>t-test (2-tailed) Significance</th>
<th>Sidak (2-tailed) Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal IQ</td>
<td>120.7</td>
<td>121.3</td>
<td>p=0.516</td>
<td>p=0.887</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>127.5</td>
<td>128.0</td>
<td>p=0.680</td>
<td>p=0.967</td>
</tr>
<tr>
<td>Full Scale IQ</td>
<td>125.5</td>
<td>126.3</td>
<td>p=0.442</td>
<td>p=0.826</td>
</tr>
</tbody>
</table>

McGuire et al. Neurol 2014;83:638-645
## MicroCog (current) Neurocognitive Differences

**Significant MicroCog differences c/w subcortical injury**

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

MicroCog values parallel WMH burden within the U2P population

Separation point DOC median value WMH count/volume

<table>
<thead>
<tr>
<th>Level</th>
<th>MicroCog (current)</th>
<th>Lower WMH Burden</th>
<th>Upper WMH Burden</th>
<th>t-test (2-tailed) Significance</th>
<th>Sidak (2-tailed) Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Count (n=36)</td>
<td>Volume (n=33)</td>
<td>Count (n=65)</td>
<td>Volume (n=68)</td>
</tr>
<tr>
<td>1</td>
<td>Attention/mental control</td>
<td>105.8</td>
<td>105.9</td>
<td>105.0</td>
<td>105.0</td>
</tr>
<tr>
<td>1</td>
<td>Reasoning/calculation</td>
<td>104.8</td>
<td>102.8</td>
<td>97.5</td>
<td>98.8</td>
</tr>
<tr>
<td>1</td>
<td>Memory</td>
<td>109.8</td>
<td>108.5</td>
<td>103.3</td>
<td>104.2</td>
</tr>
<tr>
<td>1</td>
<td>Spatial processing</td>
<td>110.4</td>
<td>110.3</td>
<td>108.3</td>
<td>108.4</td>
</tr>
<tr>
<td>1</td>
<td>Reaction time</td>
<td>108.5</td>
<td>109.6</td>
<td>107.0</td>
<td>106.5</td>
</tr>
<tr>
<td>2</td>
<td>Information processing speed</td>
<td>106.8</td>
<td>105.0</td>
<td>102.7</td>
<td>103.7</td>
</tr>
<tr>
<td>2</td>
<td>Information processing accuracy</td>
<td>105.2</td>
<td>105.3</td>
<td>100.8</td>
<td>100.9</td>
</tr>
<tr>
<td>3</td>
<td>General cognitive functioning</td>
<td>107.4</td>
<td>106.4</td>
<td>102.2</td>
<td>103.9</td>
</tr>
<tr>
<td>3</td>
<td>General cognitive proficiency</td>
<td>109.0</td>
<td>108.0</td>
<td>104.2</td>
<td>104.9</td>
</tr>
</tbody>
</table>
Pending Functional Analyses

귀중한 NP 결과를 다음으로 비교하는 경우:

- WMH burden (FLAIR)
- DTI
- DTI modulated by WMH burden
- MRS
NDCS Hypothesis

- Mechanism related to $N_2$ gas bubble release associated with decrease in ambient pressure
- Arterial macroembolization relatively uncommon
- Microembolization ($< 30 \, \mu m$) dominant mechanism
  - Possibly occurs with every exposure – intensity variable
  - Possible microemboli include gas bubbles, thrombin-platelet aggregates, or microparticles/activated proleukocytes
    - Randomly distribute to CNS proportionally to blood flow
  - Alternative hypothesis cytotoxicity with intracellular ischemia
    - Presumably without arteriolar hypoxia
- Process is not micro- or macrohemorrhagic
  - No hemosiderin deposits detected on high resolution MRI
- Predominantly a hemispheric problem, not posterior fossa or spinal cord
NDCS Hypothesis II

- Degree of FLAIR findings related to intensity of exposure
  - Reflection of duration/intensity/frequency of exposure
  - Reflection of innate capacity for recovery (biovariability)
  - No simple relationship to total hours of exposure

- Unclear whether initially an axonal/demyelinating process or a cytotoxic/intracellular ischemic process

- Cortical remodeling complex and not understood

- Neurocognitive differences reflect axonal +/- neuronal injury

- Long-term effects unclear
Unanswered Questions

- What is the pathophysiological mechanism behind WMH associated with non-hypoxic hypobaric exposure experience?
- What is the temporal course and how does this vary with exposure intensity/frequency?
- What operational modifications are appropriate?
  - Are there additional mitigation strategies we can employ?
  - What period of recovery should occur between exposures?
  - Is hyperbaric therapy alone sufficient?
  - What are the implications for repeated/frequent astronaut exposure?
- What are the long-term consequences of WMH change and neuronal loss?
USAF Operational Changes

- USAF changes
  - U-2 pilot exposure limitation AFI 11-2U-2v3, Table 7.1 revision (minimum 72 hours between 9 hour flights)
  - U-2 cockpit modification (CARE program) – completed
  - U-2 pilot occupational MRI monitoring program
  - Recommended limitation for chamber personnel exposure (minimum 72 hours between exposures)
  - Expansion of informed consent for human research
Current Research

- *Sus scrofa domestica (Sinclair mini-pig) model*
- *Single exposure study*
- *Mortality study*
- *Calibration study*
Sus scrofa domestica model

- Prototype model to demonstrate feasibility for research
- Six 8hr flights over 2 weeks
  - 30k altitude/100% $O_2$
- General anesthesia for flights/MRI
  - Neuroprotective effects of anesthesia
- Includes MRI, serological, and necropsy correlation
Sus scrofa domestica – FLAIR

Sham pig – no change in before – after FLAIR
Sus scrofa domestica – MRS (sham pig)

- MRS – sampling at both TE135 and TE30
- May reflect early injury
Examine acute (MRI/serological) changes following a single exposure – all meet FCII/FCIII neurological standards

- Hypobaric-hypoxic (traditional aircrew chamber training)
- Hypobaric (AOP inside safety monitors)
- Hypoxic (ROBD – reduced O₂ breathing device)
- Control

Protocol:

- MRI 24hr before; 24hr after; 72hr after
- Serological immediately before; immediately after; 24hr after; 72hr after
- No other altitudinal exposure beginning 7d prior
- No alcohol beginning 7d prior
- Maintain normal physiological activities

Intra-subject and cross-group comparisons
Single Exposure Analyses

- Imaging analyses:
  - FLAIR – WMH burden
  - MR spectroscopy comparison
    - Total NAA (neuronal viability and function)
    - Total Cr (energy metabolism)
    - Total Choline (membrane turnover)
    - Myoinositol (glial marker)
    - Glutamate (neurotransmission and metabolism)
    - Glutathione (oxidative stress marker)
  - DTI
  - ASL
  - Q-space
  - Inflammatory markers (TNF-α; interferon-γ; IL-6; S100B)
- Microparticle analysis
- Study start date 9/10/2014
Calibration Study

- **Challenge is cross-comparison of MRI scanners on DTI & MRS**

- **Goal: cross-calibration of RII and 59MDW MRI scanners**
  - Already have cross-calibration for FLAIR images
  - DTI and cortical mapping cross-correlation unclear
    - Impacts interpretation of U-2 pilot data
  - Study duration 7/2014 – 9/2014
Future Efforts

- Mortality study (USAFSAM – RAM project)
  - Preliminary results no difference in neuropsychiatric mortality
- Duke – Navy – USAFSAM animal model comparison
- NATO Study Group
- Norway Seminar
- UK Seminar
- USAFSAM proteomic and metabolemic studies in animal models
- NASA cooperative study?
Questions?