What do Diffusion Tensor Imaging Abnormalities after Traumatic Brain Injury Really Mean? 
Radiological-Pathological Correlations and Microdialysis Studies in Mice and Humans

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Disclosures

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• Previous research funded also by DARPA, National Football League, Cure Alzheimer’s Fund, Thrasher Foundation, & Burroughs Wellcome.

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• Royalties: sales of Concussion Care Manual (Oxford University Press)

• Conflicts of Interest: none
Introduction
What is traumatic brain injury?

- Injuries to the brain structure due to acute, external physical forces that result in impaired brain function.
- Usually result from car accidents, falls, or assault. Recent interest in blast-injuries due to wars in Iraq and Afghanistan.
- Range from mild injuries with transient loss of consciousness or confusion ("concussion") to devastating or fatal injuries.
- Results of repeated injuries can be cumulative (boxers, football players...)
A Historical Note...

- Traumatic brain injuries are among the earliest described illnesses in human history.
  - Among South African australopithecine specimens, there is an ~3 million year old skull with distinct cranial fractures that appear to have been caused by assault with an antelope humerus.
  - The *Edwin Smith Surgical Papyrus*, ~26th century B.C.E., provides a written description of impaired coordination, contralateral motor deficits, and impaired consciousness following war-time head injuries.

S. Finger *Origins of Neuroscience* 1994, p. 3-17
Modern Epidemiology of TBI

• Estimated incidence of 3.5 million new cases per year in the United States in 2009 (approximately 1% chance per year).
  • 300,000 hospitalized (moderate to severe)
  • 2,077,000 treated and released from emergency departments (‘mild’ /concussive)
  • 1,164,000 treated in outpatient facilities/offices
  • 52,695 fatalities

Trends since 1999: Similar # of fatalities, increase total diagnoses (increase from 1.5 M probably due more recognition of concussion), reduced # of hospitalizations from 500,000

• Highest risk ages 0-4, 15-24 and >75 years
• A total of 3.2 to 5.0 million Americans, 1-2% of the U.S. population—currently live with disabilities resulting from TBI.
• The single most common cause of permanent disability in young people, under age 45.
• Total costs are extremely high, estimated at $60-220 billion per year (primarily due to lost productivity)

Coronado et. al. J Safety Research 2012,
Thurman et. al. Journal of Head Trauma Rehabilitation 14 602-15 1999
Axonal injury can be the predominant neuropathological feature in many TBI patients with reduced levels of consciousness and/or impaired cognitive function during life.
Traumatic Axonal Injury is Common

**Table 2. Neuropathology**

<table>
<thead>
<tr>
<th>Patient</th>
<th>AI</th>
<th>Hypoxic/ischemic damage</th>
<th>Contusion</th>
<th>Gliding contusion</th>
<th>Hematoma (&gt;3 cm)</th>
<th>ASDH</th>
<th>ICP</th>
<th>Skull fracture</th>
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</table>

Blumbergs et al., J Neurotrauma 1995
"Since the amount of DAI was directly proportional to the severity of injury (duration of coma and quality of outcome), we conclude that axonal damage produced by coronal head acceleration is a major cause of prolonged traumatic coma and its sequelae."
The Axonal Injury Hypothesis

- Initial version: *a generalization from the findings from Gennarelli et al.*
  “Deficits resulting from traumatic brain injury are primarily caused by injury to specific axonal tracts.”

- Extended network version:
  “Deficits resulting from traumatic brain injury are primarily caused by disruption of specific neuronal networks resulting from injury to specific axonal tracts.”

However, most patients survive and do not come to autopsy.

Assessment of injury to specific axonal tracts in living human TBI patients is a key goal.
Story #1: Radiological-pathological correlations in preclinical animal models of traumatic brain injury
Diffusion Tensor Imaging (DTI)

1. Collect diffusion weighted images in six or more directions.
2. Calculate diffusion tensor for each voxel.
3. Separate parallel ($\lambda_1$, axial) from perpendicular ($\lambda_2, \lambda_3$, radial) diffusion. Calculate anisotropy.

Brain white matter: organized, myelinated axons.

A Traumatic axonal injury: simplified model

- Axonal Disruption: reduced $\lambda_1$ (axial), reduced anisotropy.
- Myelin Injury: incr. $\lambda_2, \lambda_3$ (radial), reduced anisotropy.
- Mixed Injury: greatly reduced anisotropy.

Figure adapted from M. Budde
Controlled Cortical Impact TBI in Mice

Brody et al.,
J Neurotrauma 2007

Electromagnetic, stereotaxic impact.
Tissue was stained with an antibody for Amyloid-β precursor protein (APP)
- Under normal conditions, APP traverses the length of axons
- When axons are injured, axonal transport is impaired
- APP accumulates in regions of impaired axonal transport
- ROI were chosen for each slice and used only areas of positively stained injured axons which included the entire gradient of staining

Mac Donald et al, Exper. Neurol 2007
DTI Validation in a Mouse Model

Mac Donald et al, Exper. Neurol 2007
Diffusion Tensor Imaging Reveals Abnormalities that Conventional MRI Does Not in Areas of Traumatic Axonal Injury

Mac Donald et al., Experimental Neurology 2007
Diffusion Tensor Imaging Consistently Distinguished Injured from Uninjured White Matter in Mice, where Conventional MRI Does Not.

Mac Donald et al., Experimental Neurology 2007
There is a Strong, Quantitative Correlation Between DTI Signal Change (Relative Anisotropy) and the Severity of Axonal Injury

Mac Donald et al., Experimental Neurology 2007
DTI-based Prediction of Axonal Injury in the Hippocampal Commissure...

... Confirmed using APP Immunohistochemistry

Mac Donald et al., Experimental Neurology 2007
Correlation with Axonal Injury Severity in an Animal Model

Change in Relative Anisotropy accurately reflects injury severity.

For the least severe injuries, (1.0 mm) DTI may be more sensitive than immunohistochemistry.
Varying time from injury to imaging

Acute time points studied: 4-6 hours, 24 hours, 4 days
Subacute time points studied: 7 days, 30 days
Chronic time point: 6 months (not shown)
Subacute White Matter Injury (1 week to 1 month)
Characterized by edema and demyelination

Mac Donald et al., J Neurosci 2007
Diffusion Tensor Imaging (Relative Anisotropy) Distinguished Injured from Control at both Acute and Subacute Time Points in Mice.

Mean Diffusivity Distinguished Acute from Subacute Injury in Mice. If similar in humans, this could have forensic implications...

Mac Donald et al, J Neurosci 2007
What about concussion?

Yoshi Shitaka, PhD

TBI-TBI

No obvious lesion

Shitaka et al JNEN 2011
Subtle Axonal Injury

Shitaka et al. JNEN 2011
DTI in repeated concussive TBI

Rachel Bennett, PhD

Bennett et al Neuroscience Letters 2012
Delayed Reduction in Axial Diffusivity

Bennett et al Neuroscience Letters 2012
Summary

• Diffusion Tensor Imaging detects pericontusional traumatic axonal injury in a mouse model accurately and quantitatively where conventional MRI does not.

• Diffusion tensor imaging is less sensitive to more subtle forms of axonal injury in mouse concussive TBI
Story #2: Correlations between DTI and microdialysis measures of traumatic axonal injury
Methods for Assessing Axonal Injury in the Brain of Living Humans

*Listed from most direct to least direct*

- Microdialysis measurements of axonal proteins released into the brain extracellular fluid
- Advanced imaging techniques sensitive to axonal injury (standard MRI and CT are not sensitive)
- Measurements of axonal proteins released into cerebrospinal fluid
- Measurements of axonal proteins released into blood
Microdialysis

Courtesy of CMA

Microdialysis Catheter
External Ventricular Drain

Poca et al, J Neurotrauma 2006

Brody, Magnoni et al Science 2008
Microdialysis Markers of Axonal Injury

Sandra Magnoni, MD

Magnoni et al Brain 2011
Tau vs.
Neurofilament Light Chain
using CMA-71
100 kDa nominal molecular weight cutoff catheters

Magnoni et al Brain 2011
Tau vs. other metabolic parameters

**A**
- Spearman $r = -0.87$, $p=0.00002$
- Initial 24h Mean Tau (pg/ml)
- Initial 24h Mean $A\beta_{1-42}$ (pg/ml)

**B**
- Spearman $r = -0.16$, N.S.
- % Change in Tau (61-72h vs 1-12h)
- % Change in $A\beta_{1-42}$ (61-72h vs 1-12h)

**C**
- Spearman $r = -0.61$, $p=0.016$
- Initial 24h Mean Tau (pg/ml)
- Initial 24h Mean Glucose (mM)

**D**
- Spearman $r = -0.44$, N.S.
- Initial 24h Mean Pyruvate ($\mu$M)
- Initial 24h Mean Tau (pg/ml)

**E**
- Spearman $r = 0.07$, N.S.
- Initial 24h Mean Lactate (mM)
- Initial 24h Mean Tau (pg/ml)

**F**
- Spearman $r = -0.25$, N.S.
- Initial 24h Mean Tau (pg/ml)
- Initial 24h Mean Glutamate ($\mu$M)

**G**
- Spearman $r = 0.41$, N.S.
- Initial 24h Mean Lactate (mM)
- Initial 24h Mean Lactate/Pyruvate

Magnoni et al Brain 2011
Early microdialysis predictors of 6 month clinical outcomes

**A**

<table>
<thead>
<tr>
<th>Clinical Outcome at 6 months (Glasgow Outcome Score-Extended)</th>
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<tbody>
<tr>
<td>Initial 24h Mean Glutamate (mM)</td>
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Spearman $r = -0.34$, N.S.

**B**

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<thead>
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<th>Clinical Outcome at 6 months (Glasgow Outcome Score-Extended)</th>
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<tr>
<td>Initial 12h Mean Tau (pg/ml)</td>
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Spearman $r = -0.60$, $p = 0.018$

**C**

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<th>Clinical Outcome at 6 months (Glasgow Outcome Score-Extended)</th>
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<tr>
<td>Initial 24h Mean Glucose (mM)</td>
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Spearman $r = 0.33$, N.S.

**D**

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<tr>
<th>Clinical Outcome at 6 months (Glasgow Outcome Score-Extended)</th>
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<td>Initial 24h Mean Lactate/Pyruvate</td>
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Spearman $r = -0.34$, N.S.

**E**

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<th>Clinical Outcome at 6 months (Glasgow Outcome Score-Extended)</th>
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<td>Initial 24h Mean Glutamate (mM)</td>
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Spearman $r = -0.32$, N.S.

Magnoni et al Brain 2011
Microdialysis vs. DTI Workgroup

Nino Stocchetti, Sandra Magnoni*

Christine Mac Donald,

Brian Sammons

James Sorrell

TJ Esparza
15 Severe TBI patients studied with microdialysis and diffusion tensor imaging

<table>
<thead>
<tr>
<th>ID</th>
<th>Age(^a) (years) M/F</th>
<th>GCS</th>
<th>Marshall CT Classification</th>
<th>Classification of TAI on conventional MRI(^b)</th>
<th>Surgery Y/N</th>
<th>Time to scans after TBI w/m</th>
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<td>Y + DC</td>
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## Microdialysis Data

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<th>Tau (μg/ml)</th>
<th>Glucose (mM)</th>
<th>Lactate (mM)</th>
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<td>16.5</td>
<td><strong>32.8</strong></td>
<td>26.0</td>
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</table>

*Magroni et al *Brain* 2015*
Coregistration of DTI with CT

Red arrow showing location of microdialysis catheters for analysis of white matter around the catheter sampling site.

DTI acquisition on a Philips 3T Achieva scanner with patients sedated and ventilated. 32 directions at $b=1000$ Voxel size $2 \times 2 \text{ mm}$ Slice thickness $2 \text{ mm}$ Scan time 35 min total

Masking of white matter only on DTI using a Fractional Anisotropy threshold >0.2 within a 1 cm radius sphere.

Magnoni et al *Brain* 2015
DTI in individual patients vs. individually matched controls

- For each patient, a region of interest anatomically matched to the location of the catheter was sampled in 5 age-matched controls.
- Clearly some patients have very abnormal relative anisotropy, and some are essentially normal.

Magnoni et al. *Brain* 2015
Other DTI parameters

Magnoni et al. *Brain* 2015
Microdialysis Tau vs. Normalized DTI Relative Anisotropy

Analyses of DTI blinded to tau data. Analysis of tau data blinded to DTI results

Magnoni et al. *Brain* 2015
Other DTI Regions of Interest

Magnoni et al. *Brain* 2015
Other Metabolic Markers and Amyloid-beta

Magnoni et al. *Brain* 2015
Close Correlation Between Microdialysis Tau and Normalized Relative Anisotropy on DTI

- This greatly increases our confidence of the interpretation of both methods, since it is very unlikely that two entirely distinct sets of technical caveats and noise sources would give these results.
- Abnormal DTI in other regions indicates that changes are not due to catheter insertion.
- The two methods have complementary advantages.
  - DTI provides whole brain coverage but requires patient transport and can usually be performed only a few times
  - Microdialysis provides continuous sampling, but only reflects a limited portion of the brain.
Ongoing and Future Directions

• Standardization for multicenter trials.
• Assessment of multivariate prognostic models: do measurements of axonal injury severity improve existing predictors (IMPACT, conventional MRI)?
• Pharmacodynamic assessments of candidate therapeutics targeting axonal injury using both DTI and microdialysis.
Pharmacokinetics and pharmacodynamics

Intravenously infused IL-1ra gets into the brain

Intravenously infused IL-1ra alters brain cytokine profiles

Table 4. Mean microdialysate MDC concentrations (pg/mL) ± standard error

<table>
<thead>
<tr>
<th>Time in relation to drug administration</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
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<tbody>
<tr>
<td>Control group</td>
<td>38.1 ± 11.2</td>
<td>39.0 ± 11.8</td>
<td>40.3 ± 103.4</td>
<td>44.2 ± 16.0</td>
<td>45.4 ± 17.5</td>
</tr>
<tr>
<td>Intervention group</td>
<td>8.4 ± 1.7</td>
<td>9.4 ± 2.0</td>
<td>9.6 ± 2.0</td>
<td>9.6 ± 1.8</td>
<td>1.04 ± 1.9</td>
</tr>
</tbody>
</table>

MDC: Macrophage-derived chemoattractant

Helmy et al, JCBFM 2014
Story #3: Radiological pathological correlations in human brain tissue from patients with Chronic Traumatic Encephalopathy

McKee et al., Brain 2012
The Problem

- **Chronic traumatic encephalopathy cannot be detected in living human patients**
  - Clinical signs and symptoms overlap with other conditions
  - Tau PET tracer-based methods have not been validated in CTE
  - Tau PET tracer-based methods typically have \(~4~\text{mm}\) spatial resolution and may not have adequate spatial resolution to detect \(1-2~\text{mm}\) focal tau pathology at the depths of the sulci and perivascular regions
  - No blood or CSF-based markers for CTE have been developed.
Hypotheses (work in progress: not all tested yet)

1) High spatial resolution advanced diffusion MRI may be able to detect disrupted brain microstructure due to CTE-related tau pathology.

2) Tau pathology in CTE may be closely related to traumatic axonal injury adjacent to the tau-laden depths of sulci and in tau positive perivascular regions.

3) Advanced diffusion MRI may detect the traumatic axonal injury in CTE cases and distinguish them from other TBI cases and other neurodegenerative disorders.
Approach

• Methods development for advanced diffusion MRI in fixed human tissue.

• Methods development for direct co-registration of MRI data sets distorted by magnetic field inhomogeneities and histology data sets distorted by tissue processing.

• Methods development for *quantitative* histology relevant to CTE (previous studies have been qualitative in nature)

• High resolution quantitative radiological-pathological correlation analysis in blocks of fixed brain tissue from pathologically confirmed CTE cases and relevant controls.
Advanced Diffusion MRI in Fixed Human Tissue

- 3 x 2 x 1 cm block of fixed normal control brain
- Custom MRI coil
- 11.7T small bore scanner
- Very high signal to noise

Joong Hee “Caleb” Kim et al unpublished data
Optimizing Parameters for Imaging

Optimization of Tissue Rehydration in Saline to remove aldehydes, Repetition Time, Fluorinert immersion, T1, T2, b-values (not shown).

Joong Hee “Caleb” Kim et al. *unpublished data*
Optimizing Spatial Resolution and Filtering

0.4 mm native spatial resolution DTI
30 directions, b=4000
Some “Gibbs Ringing” at tissue interfaces.
Addressed using Hamming filter

Joong Hee “Caleb” Kim et al *unpublished data*
Diffusion Kurtosis Imaging

Multiple b-values: non-linear diffusion attenuation
Spatial Resolution 0.25 x 0.25 x 0.5 mm

Joong Hee “Caleb” Kim et al *unpublished data*
Diffusion Kurtosis Imaging in normal tissue

5 b-values, 30 diffusion directions per b-value
Spatial Resolution 0.25 x 0.25 x 0.5 mm

Joong Hee “Caleb” Kim et al unpublished data
Diffusion Tensor and Generalized Q-ball Imaging

T2 Weighted Image (T2WI)

Fractional Anisotropy (FA)

Generalized Fractional Anisotropy (GFA)

1st major fiber ratio in white matter

2nd major fiber ratio in white matter

DTI with 30 directions at b=4000
GQI with 202 directions and max b=8000
Spatial Resolution 0.25 x 0.25 x 0.5 mm

Joong Hee “Caleb” Kim et al unpublished data
Test-retest reliability

Fractional Anisotropy:
Very reliably in some regions, less reliable in others

Joong Hee “Caleb” Kim et al unpublished data
Kurtosis measures even more reliable than anisotropy measures.
Coregistration of Diffusion MRI and Histology

Multiple matched anatomical landmarks placed on both histological and MRI data sets.

Affine Transformation (Cortical Tissue)

Nonlinear Moving Least Squares (Cortical Tissue)

Standard method: Low accuracy

Novel method: Higher accuracy

Mihika Gangolli et al *unpublished data*
Excellent match between fiber direction information from diffusion MRI (y axis) vs. histopathology (x axis) at the individual voxel level.

Mihika Gangolli et al *unpublished data*
Quantitative Histology

AT8 (phospho-tau) staining from a CTE brain courtesy of Dr. Ann McKee, Boston U

Warped tissue mask: Each box matched to 1 MRI voxel after correcting for distortions

Laurena Holleran et al unpublished data

VisioPharm-based quantitative analysis of tau tangles. Automated based on size, shape, and intensity in each voxel-matched region.
Laurena Holleran et al.
unpublished data

No clear correlation between diffusion parameters and tau pathology
Radiological-Pathological Correlation

Holleran, Gangolli, Kim et al *unpublished data*

**A**
- Intact axons underlying tau negative sulcus
- Disorganized axons underlying tau positive sulcus

**B**
- FA: 
  - WM Tau Neg
  - WM Tau Pos

**C**
- Generalized FA: 
  - WM Tau Neg
  - WM Tau Pos

**D**
- Lower anisotropy and axial diffusivity underlying tau positive sulcus

**E**
- Axial Diffusivity: 
  - WM Tau Neg
  - WM Tau Pos

**F**
- Radial Diffusivity: 
  - WM Tau Neg
  - WM Tau Pos

Intact axons underlying tau negative sulcus
Disorganized axons underlying tau positive sulcus
A challenge: Direct assessment of injured axons in human CTE

- Rare to no APP staining
- Erratic SMI34 (neurofilament) staining
- Negative NF-H (neurofilament heavy chain) staining
- Inconsistent silver staining in human tissue, though works very well in mouse and rat tissue.
- Most reliable has been disruption of myelin stains (myelin basic protein, myelin black gold histology)
Cortex  CTE tissue SMI34

Laurena Holleran et al
unpublished data
Quantitative Analysis of Axonal Injury

Black Gold II

Normalized and windowed

Power spectrum fit with ellipse

Curving fibers (gray/white matter boundary, tau negative sulcus)

Injured fibers (gray/white matter boundary, tau positive sulcus)

Mihika Gangolli et al *unpublished data*

Disrupted organization of myelin stain appears useful as a histological marker of chronic axonal injury

\[
1 - \frac{\lambda_2}{\lambda_1} = .760
\]

\[
1 - \frac{\lambda_2}{\lambda_1} = .371
\]
Future directions

• Testing additional diffusion parameters for direct correlations with tau pathology. *(so far, no strong direct correlations)*

• Further and more quantitative analysis of additional tissue samples from CTE patients and controls with sulcal and perivascular tau. *(promising initial findings with regard to axonal injury underlying tau positive sulci)*

• Direct electron microscopic and array tomographic evaluations of axonal integrity

• Examination of radiological-pathological correlates of gray-white junction astrocytosis (Dan Perl et al, data presented at 2015 Department of Defense Blast Injury Research Program Expert Panel)

• Assessment of radiological-pathological correlates of microgliosis

• Assessment of minimum spatial resolution and signal to noise requirements for detection of CTE-related pathologies by diffusion MRI *(extraordinarily high spatial resolution and SNR performed ex vivo is not yet feasible in vivo, but may be possible in the future)*
DTI on a High Gradient Siemens Prisma with 32 channel head coil

Laurena Holleran et al
unpublished data

Spatial Resolution = 1.25 mm$^3$
But...

• Test-retest reliability was not good at 1.25 mm spatial resolution.
• So next we tested 1.75 mm isotropic voxel size (2.7 fold larger volume)
Test-Retest validation: Normal control scanned twice, 1 week apart, 1.75mm$^3$ isotropic voxels, 30 dwi with b=1000s/mm$^2$, with AP and PA phase encoding Directions corrected using DR_BUDDI.

Laurena Holleran et al unpublished data
Freesurfer-based White Matter Automated Segmentation – 181 ROI’s listed & coded

Laurena Holleran et al
unpublished data
Test-retest reliability at 1.75 mm voxel size

- Average 3% absolute difference in FA between scan 1 and scan 2 over 181 regions of interest.
- 30/181 regions with >5% absolute difference in FA,
  - 6/77 white matter regions
  - 24/104 gray matter regions

<table>
<thead>
<tr>
<th>White Matter ROIs</th>
<th>Series1 FA</th>
<th>Series2 FA</th>
<th>Mean FA</th>
<th>Difference</th>
<th>%Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC_Posterior</td>
<td>0.8056</td>
<td>0.7943</td>
<td>0.79995</td>
<td>0.0113</td>
<td>1.412588</td>
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<tr>
<td>CC_Central</td>
<td>0.5866</td>
<td>0.5993</td>
<td>0.59295</td>
<td>-0.0027</td>
<td>-2.14183</td>
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<tr>
<td>CC_Anterior</td>
<td>0.634</td>
<td>0.6206</td>
<td>0.6273</td>
<td>0.0073</td>
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<tr>
<td>wm-lh-lateralorbitofrontal</td>
<td>0.3337</td>
<td>0.3297</td>
<td>0.3317</td>
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<td>-0.601853</td>
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<td>wm-lh-medialorbitofrontal</td>
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<td>wm-lh-parahippocampal</td>
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<td>wm-lh-paroangularis</td>
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<td>wm-lh-temporalpole</td>
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<td>0.3125</td>
<td>0.3039</td>
<td>-0.0172</td>
<td>-5.65976</td>
</tr>
</tbody>
</table>

Laurena Holleran et al unpublished data
Summary

• High spatial resolution radiological pathological correlations in human ex-vivo brain tissue are challenging but technically feasible.

• White matter disruption adjacent to sulcal depths could be related to tau pathology in Chronic Traumatic Encephalopathy.

• Spatial resolution remains a major challenge for diffusion tensor imaging. High spatial resolution comes at a cost of reduced reliability.

• Nonetheless, high gradient scanners (Prisma @ 80 mT/m) +32 channel headcoil allows very good spatial resolution and reliability for future in vivo studies of human TBI patients.

• There is an unmet medical need for very high (<0.5 mm) spatial resolution methods for assessing axonal injury in humans.
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