Long-Term White Matter Changes in a Bilateral Model of Repetitive Mild Traumatic Brain Injury

Virginia Donovan[1,2]

Jacqueline Coats[3]

Alena Mohd-Yusof[4]

Lei Huang[5]

Andre Obenaus[1,2,3,5,6]

[1]Cell, Molecular and Developmental Biology

[2] NSF IGERT Program in Video Bioinformatics

University of California, Riverside

[3]Radiation Medicine

Loma Linda University, Loma Linda

[4]Psychology Department

California State University, San Bernadino

[5]Biophysics and Bioengineering

[6]Radiology

Loma Linda University, Loma Linda

Abbreviations

ADC= Apparant Diffusion Coefficient
CCI= Controlled cortical impact
DTI= Diffusion Tensor Imaging
MRI = Magnetic Resonance Imaging
mTBI = Mild traumatic brain injury
rmTBI= Repetitive mild traumatic brain injury
T2WI = T2 weighted imaging

Acknowledgments

Doug Hauser and Ernesto Barron at the Cell and Tissue Imaging Core, University of Southern California for their assistance with TEM tissue preparation and imaging.

Sonny Kim and Kamal Ambadipudi for MRI assistance.

This work was supported by the Department of Defense, DCMRP#DR080470

IGERT: Video Bioinformatics, DGE090367

Correspondence

A. Obenaus - aobenaus@llu.edu

Introduction

Mild traumatic brain injury (mTBI) is an important public health concern because symptoms can be transient and/or subtle, making this mild injury difficult to recognize. Difficulty in identifying incidence of mTBI can lead to an increased risk for subsequent injuries, which may exacerbate developing neuropathology. Evolving white matter damage within the brain can lead to long-term neurological and cognitive deficits and longer recovery times.

In this study we explored the long-term effects of repetitive mTBI on the corpus callosum using a model in which two controlled cortical impacts were induced to opposite sides of the brain. The integrity of the corpus callosum was evaluated using diffusion tensor imaging and electron microscopy confirmed the neuroimaging findings.

Methods

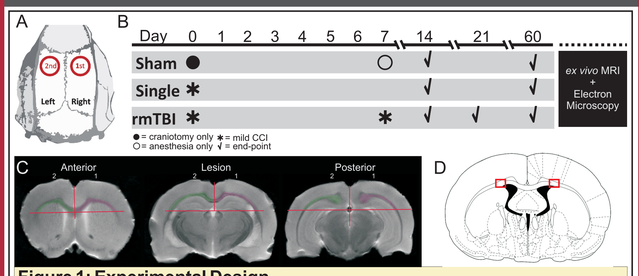

Figure 1: Experimental Design
A. Locations of the first and second mild controlled cortical impacts (mCCI). **B.** Experimental time line: on day 0 all groups received a craniotomy; mTBI groups (Single and rmTBI) also received a mCCI. Seven days later the rmTBI group received a second contralateral mCCI. Animals were sacrificed at 14, 21, or 60 day end-points and brains then underwent ex vivo analysis. **C.** Examples of anterior, lesion, and posterior corpus callosum drawn on ex vivo T2WIs, which were then copied to registered DTI scans for analysis; 1=side of 1st impact, 2=side of 2nd impact. **D.** The anatomical location, using the Paxinos & Watson rat brain atlas, at which the right and left corpus callosum was dissected (red box) for electron microscopy.

Experimental Groups:
Adult Sprague Dawely rats were randomized into three experimental groups (Fig. 1 A,B):
Sham: A single craniotomy on the right cortex at day 0.
Single: A single mCCI on the right cortex at day 0.
rmTBI: (repetitive mTBI) Identical parameters as Single, with a second impact on the left cortex at day 7 (Fig. 1A).

Controlled Cortical Impact (CCI):
The right cortical surface of the brain was exposed via a 5mm craniotomy (3mm posterior, 3mm lateral to bregma). A CCI (4mm tip diameter, 0.5mm depth at 6.0 m/s, 200ms dwell time) was induced on the cortical surface of animals within the mTBI groups (Single and rmTBI) using an electromagnetically driven piston. The second mCCI of the rmTBI group was delivered with identical impact parameters on the left cortical surface (Fig. 1A).

Magnetic Resonance Imaging (MRI):
Animals for MRI analysis were sacrificed on day 14, 21, or 60 via 4% paraformaldehyde perfusion. White matter changes were evaluated on ex vivo brains using T2 weighted images (T2WI) and diffusion tensor images (DTI) collected on a 11.7T (Bruker Biospin). Regions of interest (ROI) included the corpus callosum, within 3mm anterior and posterior of the T2WI slice directly under the site of impact (Fig. 1C). ROIs were then transferred to registered DTI scans where radial, axial, and ADC measurements were extracted. Extracted measurements were grouped together for slices anterior to, posterior to, and under the impact site [lesion]. Experimental measurements are presented as percent change from Sham 14 days post injury, which had ADC values of 0.813±0.063 (in 10^{-3} mm^2/s), similar to those previously shown in normal adult rat corpus callosum [data not shown].

Electron Micrographs:
Animals for electron microscopy analysis were sacrificed on day 14, 21, or 60 via 2% gluteraldehyde perfusion. Blocks measuring $2mm^3$ were dissected from the left and right body of the corpus callosum, 2mm anterior to the slice centered under the impact site (Fig. 1D). Tissue was embedded in epon and imaged using a JEOL JEM-2100 LaB6 Transmission electron microscope.

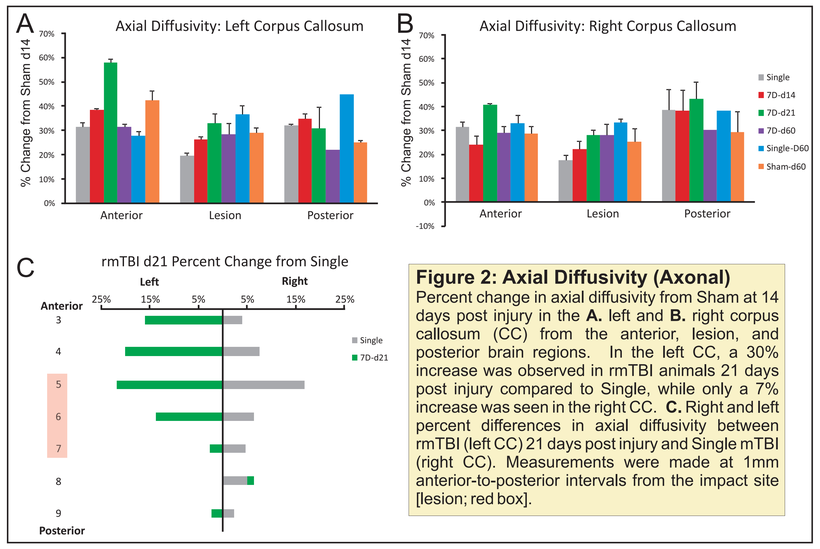

Figure 2: Axial Diffusivity (Axonal)
Percent change in axial diffusivity from Sham at 14 days post injury in the **A.** left and **B.** right corpus callosum (CC) from the anterior, lesion, and posterior brain regions. In the left CC, a 30% increase was observed in rmTBI animals 21 days post injury compared to Single, while only a 7% increase was seen in the right CC. **C.** Right and left percent differences in axial diffusivity between rmTBI (left CC) 21 days post injury and Single mTBI (right CC). Measurements were made at 1mm anterior-to-posterior intervals from the impact site [lesion; red box].

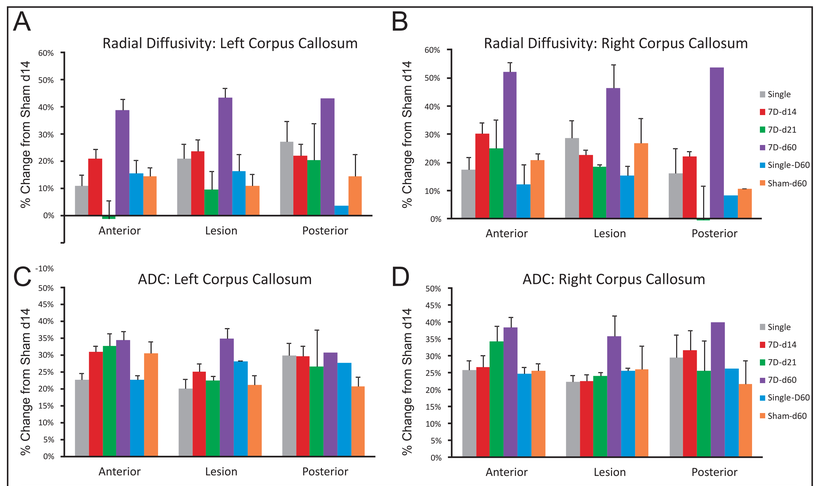

Figure 3: Radial (Myelin) and Mean Diffusivity (ADC)
Radial diffusivity in the **A.** left and **B.** right corpus callosum (CC) reveled a global increase within rmTBI animals at 60 days post injury, while Single mTBI values at 60 days post injury returned to 14 days post injury levels. Apparent diffusion coefficients (ADC) within the **C.** left and **D.** right CC reveals increased water mobility in rmTBI animals at the site of impact (lesion) at 60 days post injury in all regions.

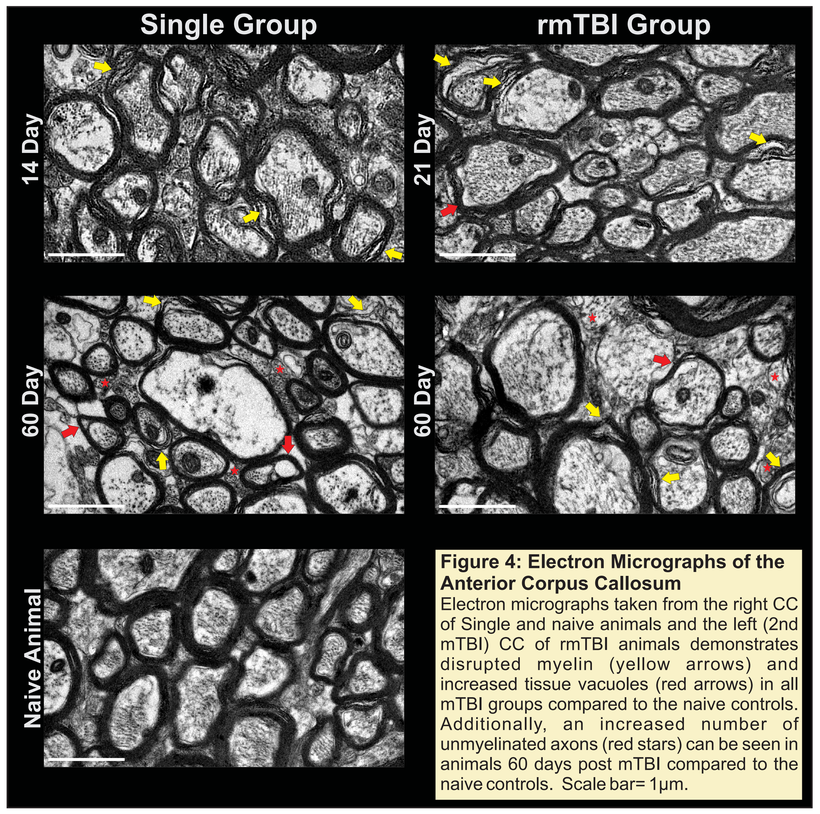

Figure 4: Electron Micrographs of the Anterior Corpus Callosum
Electron micrographs taken from the right CC of Single and naive animals and the left (2nd mTBI) CC of rmTBI animals demonstrates disrupted myelin (yellow arrows) and increased tissue vacuoles (red arrows) in all mTBI groups compared to the naive controls. Additionally, an increased number of unmyelinated axons (red stars) can be seen in animals 60 days post mTBI compared to the naive controls. Scale bar= 1µm.

Results

Axial Diffusivity (Axonal): Axial diffusivity measurements from the anterior corpus callosum (CC) revealed a bilateral increase within the rmTBI group at 21 days post injury compared to other groups (Fig. 2 A,B). The diffusivity in the left anterior CC (2^{nd} injury) of this group was approximately 25% larger than that seen in the injured [right] CC of Single animals within the anterior region adjacent to the site of impact [lesion region] (Fig. 2C). A smaller 5% increase was seen within the right anterior CC of rmTBI animals compared to Single controls (Fig. 2B). However, the increased axial diffusivity within the rmTBI group resolved by 60 days post injury, as diffusivity returned to that seen in the Single group [Fig. 2 A,B].

Radial Diffusivity (Myelin): A global 20-30% increase in radial diffusivity was seen in the CC of rmTBI animals at 60 days post injury, which was not seen within the Single or Sham groups 60 days post injury (Fig. 3 A,B). Within the left and right CC, no significant diffusivity changes were seen between Single and rmTBI groups (with the exception of rmTBI at 60 days) and Sham animals at 60 days post injury (Fig. 3 A,B).

Mean Diffusivity (ADC): ADC in the left CC revealed little change between groups in all regions, except for a 5% increase within the lesion CC of rmTBI animals 60 days post injury compared to Single controls (Fig. 3C). Similarly, in the right CC of lesion and in posterior regions a modest difference between groups was seen, with the exception that the rmTBI group at 60 days post injury had increased ADC values compared to other groups (Fig. 3D). The corpus callosum within the anterior regions showed a bilateral increase in ADC values within the rmTBI groups at sub-acute (14, 21 days) and chronic (60 days) time points, which was not seen in Single controls [Fig. 3 C,D].

Electron Microscopy: Electron micrographs from the right CC of Single and Naïve controls and left CC (2^{nd} impact) of rmTBI animals demonstrated increased myelin disruption, appearing as frayed myelin sheaths and vacuoles within the CC were seen in all Single and rmTBI animals. At 60 days post injury, an increase in the number of unmyelinated axons was seen within the anterior CC

Conclusions

- The increased axial diffusivity seen within the anterior corpus callosum (CC) of rmTBI animals at 21 days post injury suggests that this region of the CC remains vulnerable to axonal injury as a result of repetitive mTBI 7 days apart.

- While axonal damage appears to resolve by 60 days post injury, the increased radial diffusivity measurements suggest that a second mTBI results in ongoing myelin damage at later time points, which is not observed in Single mTBI animals.

- mTBI appears to result in increased unmyelinated axons, vacuoles, and frayed myelin sheaths demonstrating changes in myelin and axonal integrity.

- Thus rmTBI results in increased white matter damage, compared to Single mTBI events.

UNIVERSITY of CALIFORNIA
Riverside

LOMA LINDA UNIVERSITY

ENGINEERING LIFE SCIENCES
NSF INTEGRATED GRADUATE EDUCATION, RESEARCH AND TRAINING (IGERT) PROGRAM IN
VIDEO BIOINFORMATICS
UNIVERSITY OF CALIFORNIA, RIVERSIDE