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Diffusion MR “Microscopy” of long-term microstructural changes in the mouse cortex following CCI.

#### Introduction:

Recent advances in the acquisition and processing of fixed tissue MRI have made possible quantitative imaging of brain tissue with previously unattainable resolution and quality. So called “MR-microscopy” techniques have several advantages over conventional pre-clinical approaches for understanding neurological disorders including: whole-brain coverage, high-throughput quantitative analysis and the potential for direct translation to human disorders. The application of diffusion MR microscopy and in particular diffusion tensor MRI (DTI) is particularly suited to the investigation of microstructural brain abnormalities that are caused by traumatic brain injury (TBI) and the complex trajectory of tissue change that evolves in the days, weeks and months following injury. In this initial “proof-of-concept” study, we have optimized diffusion MR microscopy for analysis of the injured mouse brain and have demonstrated new DTI markers of brain structure abnormalities following controlled cortical impact (CCI) that offer insight into the complexity of post-traumatic tissue change.

#### Methods:

*Mouse brain CCI* - Mild CCI was performed in a mouse by standard procedures with bit size = 1.5 mm, velocity = 1.5 m/s, dwell time = 100 ms and penetration depth = 1 mm. Four weeks following CCI, the mouse underwent transcardial perfusion with 4% PFA and the brain was removed. For comparison, the brain of a surgery-naive control mouse was obtained in the same manner. Brains were stored in 4% PFA and at least 48 hours prior to imaging they were rinsed in PBS. For imaging, brains were immersed in Fluorinert (FC-77, 3M, St. Paul, MN) and held in a 10mm diameter NMR tube.

*Diffusion MR “microscopy”* - Imaging was performed on a 7T Bruker Avance-III microimaging spectrometer using a 10mm diameter linear coil and operating paravision software (v5.0.1) to acquire 452 3D-EPI image volumes with the following parameters - TE/TR=32/700ms, 8 segments, 100um<sup>3</sup> isotropic resolution, with double-sampling and trajectory mapping applied (based on Yee et al., 2011). Five non-weighted (b=0) volumes were acquired and the following diffusion weighting parameters of b-values in s/mm<sup>2</sup> and number of non-colinear diffusion gradient directions (given in parentheses) =400(14), 1700(32), 3800(32), 6500(56) and 10,000(87). Two repetitions of a single average for each image were acquired. Total scan time was approximately 63 hours. DTI post-processing - including rigid-body registration, non-linear tensor fitting and DTI index mapping - was performed using the TORTOISE pipeline (Pierpaoli et al., 2010) for

each b-value separately as well as for the entire data set. Region of interest (ROI) analysis was performed using manually drawn masks of the perilesional, frontal and visual cortex on the side of the CCI lesion as well as corresponding areas on the uninjured side.

#### Results:

*Image quality.* For the high-resolution data sets (100  $\mu\text{m}$  isotropic) diffusion tensor fitting was achieved even for very large b-values (10,000  $\text{s/mm}^2$ ), where the diffusion weighted MRI signal is greatly attenuated. The Henkelman (Henkelman, 1985) signal to noise ratio for an ROI in the thalamus in the non-weighted image was 25 and signal in all regions of brain tissue in the most diffusion weighted and attenuated images ( $b=10,000 \text{ s/mm}^2$ ) were greater than noise.

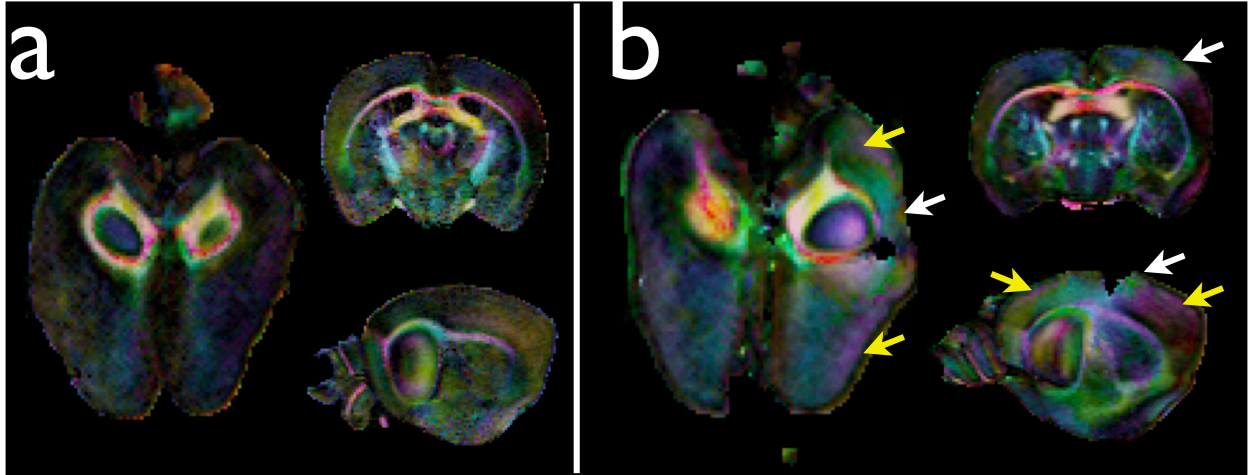
*DTI markers of structural abnormalities.* The resulting DTI maps were found to accurately represent the known microstructural properties of the mouse brain and to reveal robust patterns of tissue abnormalities in the injured brain including:

- 1) Perilesional increases in FA - A region of tissue surrounding the CCI cavity approximately 1 mm in all directions was observed to have abnormally increased FA. The edges of this tissue region are sharply discernible from surrounding brain areas and the FA within the perilesional region was 71% greater ( $\text{FA}_{\text{ipsi}}=0.318$  and  $\text{FA}_{\text{contra}}=0.183$  for  $b=1700 \text{ s/mm}^2$ ) than that of the contralateral side and control brain values. The direction of the primary eigenvalue, which is an indication of tissue orientation was also found to be different from surrounding cortex. Normally this orientation is perpendicular to the cortical surface, but in the peri-lesional tissue the primary orientation was perpendicular to the lesion cavity wall.
- 2) Distant cortical DTI changes - FA was also elevated on the side of injury in cortical regions outside of the peri-lesional area. When compared with the contralateral side (at  $b=1700 \text{ s/mm}^2$ ), FA was 16% increased in the frontal cortex ( $\text{FA}_{\text{ipsi}}=0.217$  and  $\text{FA}_{\text{contra}}=0.187$ ) and 81% increased in the visual cortex ( $\text{FA}_{\text{ipsi}}=0.271$  and  $\text{FA}_{\text{contra}}=0.103$ ). This difference was also accompanied by a change in the primary tissue orientation.

#### Conclusion:

High-resolution ex-vivo DTI was obtained in the mouse brain 4 weeks following CCI and DTI abnormalities were observed in the perilesional cortex as well as distant cortical regions on the side of injury. Ongoing and future work will address the reproducibility and characterization of these abnormalities. A key question to address is which type of cells, glial or neuronal, are determining the measured diffusion changes, and if the changes are related to degeneration of existing structures or sprouting of new processes. In order to better understand the underlying cellular changes of the observed abnormalities, imaging will be compared to histological analysis. In order to determine the nature of the observed abnormalities and how they may arise and progress following CCI, additional brains taken at variable times following CCI will be imaged using this protocol. In all, high-resolution DTI has been demonstrated as a potentially powerful approach to understanding the presence and nature of tissue changes following TBI.

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**Figure** Hi-resolution DTI maps of control (a) and CCI injured (b) mouse brains. DEC maps show fractional anisotropy (brightness) and tissue orientation (color), which are abnormal for the cortex of the CCI injured brain on the side of injury both in the perilesional area (white arrows) as well as in more distant cortical regions (yellow arrows).