Author/s:
Zamani, A;O'Brien, TJ;Kershaw, J;Johnston, LA;Semple, BD;Wright, DK

Title:
White matter changes following experimental pediatric traumatic brain injury: an advanced diffusion-weighted imaging investigation

Date:
2021-01-07

Citation:
Zamani, A., O'Brien, T. J., Kershaw, J., Johnston, L. A., Semple, B. D. & Wright, D. K. (2021). White matter changes following experimental pediatric traumatic brain injury: an advanced diffusion-weighted imaging investigation. Brain Imaging and Behavior: an international journal, 15 (6), https://doi.org/10.1007/s11682-020-00433-0.

Persistent Link:
https://hdl.handle.net/11343/258853

# White Matter Changes Following Experimental Pediatric Traumatic Brain Injury: An Advanced Diffusion-Weighted Imaging Investigation

Akram Zamani[1], Terence J. O'Brien[1], Jeff Kershaw[2], Leigh A. Johnston[3], Bridgette D. Semple[1#] and David K. Wright[1 # *]

[1] Department of Neuroscience, Central Clinical School, Monash University, Melbourne, VIC, 3004, Australia

[2] Applied MRI Research, National Institute of Radiological Sciences, QST, Chiba, Japan.

[3] Melbourne Brain Centre Imaging Unit and Department of Biomedical Engineering, The University of Melbourne, Parkville, VIC, 3010, Australia

Author Note

# Co-senior authors

*To whom correspondence should be addressed:

David K. Wright, Department of Neuroscience, Central Clinical School, Monash University, The Alfred Centre, 99 Commercial Road, VIC 3004, Australia, david.wright@monash.edu

Phone: +61 3 9903 0140 Fax: +61 3 9903 0556

Original Research Article, Word count: Abstract (250) Body (3281)

**Abstract**

Pediatric traumatic brain injury (pTBI) is a major community health concern. Due to ongoing maturation, injury to the brain at a young age can have devastating consequences in later life. However, how pTBI affects brain development, including white matter maturation, is still poorly understood. Here, we used advanced diffusion weighted imaging (DWI) to assess chronic white matter changes after experimental pTBI. Mice at post-natal day 21 sustained a TBI using the controlled cortical impact model and magnetic resonance imaging (MRI) was performed at 6 months post-injury using a 4.7 T Bruker scanner. Four diffusion shells with 81 directions and b-values of 1000, 3000, 5000, and 7000 s/mm$^2$ were acquired and analyzed using MRtrix3 software. Advanced DWI metrics, including fiber density, fiber cross-section and a combined fiber density and cross-section measure, were investigated together with three track-weighted images (TWI): the average pathlength map, mean curvature and the track density image. These advanced metrics were compared to traditional diffusion tensor imaging (DTI) metrics which indicated that TBI injured mice had reduced fractional anisotropy and increased radial diffusivity in the white matter when compared to age-matched sham controls. Consistent with previous findings, fiber density and TWI metrics appeared to be more sensitive to white matter changes than DTI metrics, revealing widespread reductions in fiber density and TWI metrics in pTBI mice compared to sham controls. These results provide additional support for the use of advanced DWI metrics in assessing white matter degeneration following injury and highlight the chronic outcomes that can follow pTBI.

Keywords: pediatric TBI, white matter, fiber density

## Introduction

In humans, pediatric traumatic brain injury (pTBI) is one of the leading causes of death and long-term disability in children (Faul et al., 2010). The initial insult is caused by external forces to the brain that can result in axonal injury, contusions and hemorrhages. Cascades of secondary pathophysiological events then follow in the hours, days and months post-injury. After pTBI, these slowly evolving cascades can have irreversible consequences on the brain as it undergoes maturation (Potts et al., 2006). As such, brain insult sequelae not only disrupt established neuronal networks, but can also alter or impede the formation of new networks (Giza & Prins, 2006).

In moderate and severe cases of pTBI in clinical populations, loss of tissue integrity and regional atrophy have been observed in the cortex, corpus callosum, amygdala and hippocampus (Merkley et al., 2008; Wilde et al., 2007). Furthermore, these pathological changes have been associated with behavioral dysfunction, including attention deficits, memory deficits, slowed processing speed and mood swings (Anderson, et al., 2005; Ryan et al., 2014; Wozniak et al., 2007). Such deficits can linger or develop for years after injury, for example, previous studies have reported behavioral deficits up to 35 years post-injury, with more extensive deficits becoming apparent earlier following severe injuries (Anderson et al., 1995; Anderson et al., 2004; Ryan et al., 2016). However, how pTBI affects brain development, including white matter maturation, remains poorly understood.

Magnetic resonance imaging (MRI), and in particular, diffusion-weighted imaging (DWI), can detect changes in microstructural connectivity that result from a brain injury, and potentially provide insight into the behavioral dysfunction that can occur following pTBI (Suskauer & Huisman, 2009; Wright, Liu, et al., 2017; Wright, O'Brien, Shultz, & Mychasiuk, 2017). To date, diffusion tensor imaging (DTI) has been routinely employed for assessing the white matter (Arfanakis et al., 2002; Benson et al., 2007; Huisman et al., 2004).

The most common DTI metrics used across preclinical and clinical research are fractional anisotropy (FA), and mean diffusivity (MD) or apparent diffusion coefficient (ADC). Preclinical studies have shown a transient increase in FA in the acute stages following injury due to increased inflammation and axonal swelling (Newcombe et al., 2007; Norenberg et al., 2004). Conversely, in the subacute and chronic stages, axonal tear and shear, capillary disruption, Wallerian degeneration, and loss in myelination have been hypothesized to result in reduced FA (Kumar et al., 2009; Palacios et al., 2011; Wilde et al., 2012). Other DTI metrics, including axial diffusivity (AD) and radial diffusivity (RD), have been suggested to provide greater understanding of the underlying tissue damage. For example, it has been shown that RD is related to myelin sheath integrity, and AD to fiber integrity (Mac Donald et al., 2007; Song et al., 2003).

However, the presence of multiple fiber bundles in a voxel can confound the diffusion tensor (Frank, 2002), limiting its utility as a biomarker of white matter injury. New advances in DWI processing attempt to overcome this limitation by enabling the assessment of individual fiber bundles within each voxel (known as a 'fixel'). Recently, new methods that account for both microstructural and macrostructural changes to white matter have been developed. Fixel-based morphometry provides measures of fiber density, fiber-bundle cross-section and a combined measure of fiber density and cross-section (Raffelt et al., 2017).

Changes in fiber density reflects changes in the volume of the intra-axonal restricted compartment. Numerous DWI models, including AxCaliber (Assaf et al., 2008) and neurite orientation dispersion and density imaging (NODDI) (Zhang et al 2012), have been developed to interrogate this compartment. Decreased signal from this compartment is thought to reflect a loss of axons. The space previously occupied by these axons may subsequently be filled with cell debris, or inflammatory cells with no change in the fiber bundle cross-sectional area. Alternatively, degenerating axons may result in increased extra-

cellular space (i.e. atrophy of the fiber bundle and reduced fiber cross-section) as has been observed in neurodegenerative diseases (Raffelt et al., 2017). Additionally, delayed development may result in reduced fiber cross-sections without reduced fiber density (Malhotra et al., 2019). Importantly, and in contrast to other methods, fixel-based metrics allow individual fiber bundles to be assessed, including those that cross or 'kiss', such as the corticospinal tract and superior longitudinal fasciculus (Tournier et al 2011).

Raffelt and colleagues (2017) have shown that these new metrics provided distinct information on white matter pathology. Investigating a cohort of temporal lobe epilepsy patients with a history of hippocampal sclerosis, the authors showed significantly reduced fiber density, fiber cross-section, and combined fibre density and cross section, in comparison with healthy controls, suggesting that these measures may also be useful in the context of brain injury.

Track-weighted imaging (TWI) is another recent DWI technique that characterizes properties of the streamlines passing through each voxel, such as their length (i.e. as in average pathlength mapping; APM), their mean curvature (MC), or number (i.e. as in track density imaging; TDI). The contribution of all streamlines to a voxel can then be summed or averaged to generate novel contrasts. Furthermore, as there are numerous streamlines traversing each voxel, images can be generated with increased spatial resolution (Calamante et al., 2010; Calamante, Tournier, Smith et al., 2012). We have previously shown that TWI metrics, including MC and TDI, may also be more sensitive than DTI metrics for assessing white matter changes post-TBI (Wright, Johnston, et al., 2017).

Here, we investigated the potential for these DWI metrics to provide insight into chronic white matter changes following TBI to a brain that is still developing, and compared them to traditional DTI metrics. We hypothesized that pTBI would result in chronic white

matter changes and that fixel-based and TWI metrics would prove more sensitive than traditional DTI metrics for assessing white matter change.

## Methods

### *Experimental groups*

Experiments were conducted at the University of Melbourne according to the Australian Guidelines for the Care and Use of Laboratory Animals, and with approval by the institutional Animal Ethics Committee. Mice were randomly assigned to severe TBI (n = 10) or sham groups (n = 7).

### *Animal model*

Male C57bl/6 mice were group-housed in individually ventilated cages at the Animal Resource Centre, Florey Institute of Neuroscience and Mental Health. Animals were maintained under 12 hours of continuous light per day with *ad libitum* access to food and water. Controlled cortical impact at p21 +/- 1 d (~10 g) was performed as previously described (Pullela et al., 2006; Semple et al., 2015). Briefly, pups were anesthetized under 4% isoflurane and transferred to 1.5% isoflurane, maintained throughout the surgery. A craniotomy was performed on the left parietal bone. Injury parameters were 4.5 m/s velocity, 1.7 mm depth and 150 ms duration, using a 3 mm diameter impactor tip. Sham control surgery underwent the same surgical preparation procedures but did not receive an impact.

### *MRI acquisition*

Mice were transcardially perfused at 6 months post-TBI as previously described (Semple et al., 2017). Brains were collected and fixed in 4% paraformaldehyde overnight, rinsed with PBS and embedded in 3% agar for MRI scanning with a 4.7 Tesla MRI. The scanning was performed as previously described (Semple et al., 2017). Briefly, DWIs were acquired with a cryogenically cooled surface coil using a 3-shot, 2D echo-planar imaging

protocol with the following parameters: repetition time = 11 s; echo time = 69 ms; slice thickness = 180 μm; and resolution = 180 x 180 x 180 $\mu m^3$. Four diffusion shells were acquired, each with 81 directions and b-values = 1000, 3000, 5000, and 7000 $s/mm^2$.

*MRI analysis*

A multi-shell, multi-tissue constrained spherical deconvolution (CSD) analysis was performed using MRtrix3 software. Average response functions were estimated for white matter, grey matter and CSF using 3-tissue CSD (Dhollander et al., 2018). Fiber orientation distributions (FODs) were estimated using the white matter and CSF response functions. Joint bias field correction and global normalization was performed prior to generating white matter FOD template images for both TBI and sham cohorts. The two cohort templates were then combined into a study-specific template image to which all of the subjects were registered. Three metrics were derived from the registered FODs: fiber cross-section, i.e. the cross-sectional size of the fiber bundle; fiber density, i.e. the apparent density of fibers in the bundle; and fiber density and cross-section, which reflects both the apparent density of the fiber bundle and its cross-section (Raffelt et al., 2017).

TWI was performed by generating whole-brain tractograms using 3 million streamlines with constraints on angle (22.5°), length (min = 0.5 mm, max = 15 mm) and FOD amplitude (0.06). Tractograms were registered to the study-specific template image to normalize both the length and spatial location of streamlines (Pannek et al., 2011; Wright, Johnston, et al., 2017) and three TWIs were generated from the registered tractograms: APM, MC, and TDI (Calamante et al., 2010; Pannek et al., 2011; Wright, Johnston, et al., 2017). DTI metrics were also registered to the study-specific template image for whole-brain voxel-wise statistical testing (Smith & Nichols, 2009; Wright, Johnston, et al., 2017).

Fixel-based morphometry was performed using connectivity-based fixel enhancement with 5,000 permutations and family-wise error correction (Raffelt et al., 2015). DTI and TWI metrics were also assessed using *a priori* region of interest (ROI)-based analysis. The ipsilateral and contralateral corpus callosum were traced on the study-specific FA template and the mean value for each metric determined using MRtrix3 software.

*Statistical Analysis*

Statistical testing of fixel-based metrics was performed using connectivity-based fixel enhancement (Raffelt et al., 2015) with 5,000 permutations and full correction for family-wise error (FWE). TWI and DTI metrics were also assessed using whole-brain voxel-wise statistical testing with 5,000 permutations and threshold-free cluster enhancement (Smith & Nichols, 2009; Wright, Johnston, et al., 2017). Group differences in ROI-based measures were assessed using a two-tailed Student t-test. A $p$-value $< 0.05$ was considered significant.

## Results

*Whole brain voxel-wise statistical testing revealed chronic damage to major white matter structures after pTBI*

Nonparametric permutation testing of whole brain TWI and DTI metrics revealed significant changes in major white matter tracts at 6 months following pediatric TBI (Figure 1). However, of the DTI metrics investigated, only FA showed a significant reduction in predominantly ipsilateral subcortical white matter values close to the injury site (FWE corrected $p < 0.05$; Figure 1a).

Analysis of the TWI metrics revealed that mice which sustained a TBI at pediatric age have significantly reduced TDI in the ipsilateral corpus callosum compared to sham mice (FWE corrected $p < 0.05$; Figure 1b). TBI mice also showed a bilateral reduction in APM (Figure 1c), predominately in the corpus callosum, and in MC (Figure 1d), with changes in MC also extending to deeper structures including the ipsilateral fimbria and external capsule.

[Insert Figure 1 here]

Fixel-based analysis revealed a significant decrease in ipsilateral corticospinal tract fiber density in the TBI group compared to sham animals (FWE corrected $p < 0.05$; Figure 2); However, there were no observed differences in fiber cross-section or the combined fiber density and cross-section measure.

[Insert Figure 2 here]

*A priori ROI-based analysis revealed chronic microstructural changes in the corpus callosum following pTBI*

An *a priori* ROI-based analysis of the corpus callosum was performed for each of the DTI and TWI metrics (Figure 3). We found reduced FA ($p = 0.003$; Figure 3a) and increased RD ($p = 0.010$; Figure 3b) in the ipsilateral corpus callosum in the injury group when compared to sham animals. Analysis of FA also showed a trend towards significance in the contralateral hemisphere, with the injured group having lower FA values than the controls ($p = 0.052$; Figure 3a). No differences were seen between groups for any of the other DTI metrics (data not shown).

[Insert Figure 3 here]

An *a priori* ROI-based analysis of the TWI metrics (Figures 3c-e) indicated shorter streamlines in the corpus callosum of the injury group compared to the sham group, as reflected by a decrease in APM in the ipsilateral hemisphere ($p = 0.0002$) and extending to the contralateral hemisphere ($p = 0.005$). In addition, the corpus callosum showed reduced TDI ($p = 0.0001$) and MC ($p = 0.0002$) in the ipsilateral hemisphere of injured mice when compared to shams controls. Together, these findings demonstrate persistent and widespread white matter damage in the chronic phase following experimental pediatric TBI.

## Discussion

Using whole brain and *a priori* ROI analyses, this study revealed the chronic impact of an experimental TBI during early postnatal brain development. Whole brain analysis of diffusion metrics found evidence of white matter changes in the ipsilateral corpus callosum, as well as the external capsule, fimbria and corticospinal tract, and contralateral corpus callosum. Furthermore, our ROI analysis showed that pediatric mice given an experimental TBI also had reduced FA and increased RD in the corpus callosum at 6 months after injury. These changes were complemented by a robust reduction in TDI, APM and MC. Using a combination of DTI and advanced diffusion processing methods, this study elucidated novel insights into the chronic neurodegeneration of major white matter fiber bundles that follows a pediatric TBI.

Our results in this experimental model are consistent with the majority of clinical pTBI cases that report chronic reductions in FA in major white matter bundles (Ewing-Cobbs et al., 2008; Genc et al., 2017). These studies show damage for many years after injury and suggest that subcortical white matter fibers are particularly vulnerable to injury and progressive neurodegeneration over time. Other preclinical imaging studies using the controlled cortical impact model, predominantly in the adult brain have also shown progression of neuropathology in the months following injury (Harris et al., 2016; Kamper et al., 2013). While FA is regularly reported to decrease chronically post-injury, changes in other DTI metrics have been inconsistent (Harris et al., 2016; Kamper et al., 2013; Robinson et al., 2016). However, AD and RD have been shown to correlate with histological stains for axonal damage and demyelination markers, respectively (Mac Donald et al., 2007; Song et al., 2003), and such pathology may also explain our observed increase in RD.

Irrespective of the method of analysis used, our data showed a reduction in FA in the corpus callosum, a fiber bundle that is highly vulnerable to injury due to its location and size (Levin et al., 2000). Whole brain analysis also revealed a reduction in FA in close proximity to the injury site in the unilateral parietal cortex; but it was unable to fully capture the extent and progression of injury to other brain regions. By incorporating advanced DWI metrics including TWI and fixel-based morphometry, we were able to detect a greater spread of white matter changes following injury.

TWI uses properties derived from whole brain streamlines to generate different image contrasts. Here, we investigated streamline length (APM), density (TDI) and curvature (MC) and found reductions in each metric following injury when compared to age-matched sham controls. Furthermore, whole brain analysis showed that TWI changes extended from the ipsilateral corpus callosum to the contralateral hemisphere, as well as other white matter tracks such as the external capsule and fimbria. It is important to consider that, as TWI interrogates properties of streamlines which travel throughout the brain, changes to the TWI metrics may actually reflect remote white matter injury. For example, axotomy in the ipsilateral corpus callosum will result in the early termination of streamlines and therefore, potentially a reduction in streamline density (i.e. TDI) in the contralateral hemisphere. However, both the fimbria and the external capsule have previously been shown to undergo damage in a diffuse injury model (Wortman et al., 2018), and our data here suggests that white matter damage in these structures persists to at least 6 months post-pTBI.

Consistent with earlier work, we also found that the TWI metrics—in particular, MC—appear to be more sensitive than the DTI metrics in detecting changes in white matter following injury (Wright, Trezise, et al., 2016). While it remains to be elucidated how the TWI metrics relate to the underlying pathology, recent work by Nigro et al. (2018) provides some insights. The authors investigated TDI in progressive supranuclear palsy, a rare

neurodegenerative disease characterized by abnormal tau pathology. They found significant and widespread reductions in TDI, including in the corpus callosum, bilateral corticospinal tracts and the superior cerebellar peduncle. The latter region corresponded with pathological examinations showing demyelination and tau accumulation as is also seen in TBI (Armstrong et al., 2016; Flygt et al., 2013; Mac Donald et al., 2007; Song et al., 2003; Wright, O'Brien, et al., 2017). As such, it is likely that the axonal damage and demyelination thought to be driving the observed increases in RD may also explain our observed changes in the TWI metrics.

Fiber density and fiber cross-section permit the investigation of both microstructural and morphological changes to the white matter. Previous studies have shown that these metrics, and in particular fiber density, are sensitive to white matter degeneration, both in patients with motor neuron disease (Raffelt et al., 2015) and also following experimental TBI in the adult rodent (Wright, Johnston, et al., 2017; Wright, Liu, et al., 2016). Furthermore, by investigating both microstructural and morphological changes, fixel-based metrics provide a better understanding of white matter pathophysiology. In this study, we report for the first time a reduction in fiber density along the corticospinal tract following pTBI. Although we were unable to perform histological analysis of these brains here, previous work has highlighted degeneration of the corticospinal tract following experimental TBI (Wright, Liu, et al., 2016). Interestingly, we found no changes in fiber cross-section, or the combined fiber density and cross-section metric, which may be due to the considerable development and maturation of white matter tracts that is still ongoing.

A limitation of the current study is that we have only considered a single chronic time point post-injury. While this is of crucial importance given that functional/neurobehavioral deficits persist and emerge chronically in TBI patients, it is unclear from our data whether the observed changes are the result or combination of persisting damage, progressive

degeneration, or possible retardation of growth in our TBI cohort. As such, longitudinal imaging studies, particularly in the context of a brain that is still undergoing maturation, are an important future endeavor. Furthermore, future studies should incorporate cellular, histological and behavioral analyses to elucidate how the findings relate to the underlying white matter pathology and how these changes evolve over time.

Multi-shell acquisitions, like the one used here, can also facilitate estimations of diffusion kurtosis. Diffusion kurtosis parameters characterize the non-Gaussian properties of water diffusion behavior in DWI and can provide additional information, such as estimates of intra-voxel fiber crossings (Lazar et al., 2008; Jensen et al., 2014), to complement DTI (Lu et al., 2006). Additional studies comparing diffusion kurtosis, DTI, TWI, and the fixel-based metrics investigated here, may provide further insights into the white matter degeneration that follows TBI. Finally, the methods employed in this study can also be applied in the clinical setting using routine protocols at 3T but with lower angular resolution and b-value (e.g. 27 directions at 1000 s/mm$^2$; Nigro et al., 2018) than that used here.

## Conclusion

To our knowledge, this is the first study to use advanced DWI analyses to reveal the chronic impact of an experimental TBI during early postnatal brain development. Whole brain and *a priori* ROI-based analyses revealed significant differences in fiber density, TWI, and DTI metrics in the white matter of mice 6 months after TBI. Major white matter bundles including the corpus callosum and corticospinal tract, as well as the fimbria and external capsule all showed evidence of microstructural changes. Furthermore, and consistent with earlier studies, advanced DWI metrics, including fiber density and TWI, appeared to be more sensitive to white matter changes than DTI metrics, providing additional support for their use in assessing white matter degeneration following brain injury.

**Acknowledgements**

The authors acknowledge the facilities, and the scientific and technical assistance of the National Imaging Facility at the Florey Institute of Neuroscience and Mental Health, Parkville.

**Author Contributions**

Author contributions included conception and study design (DKW and BS), data acquisition (DKW), statistical analysis (DKW and AZ), interpretation of results (All authors), drafting the manuscript work or revising it critically for important intellectual content (All authors) and approval of final version to be published and agreement to be accountable for the integrity and accuracy of all aspects of the work (All authors).

**Funding Sources**

This work was supported by the National Health and Medical Research Council to BS [R. D. Wright Biomedical Career Development Fellowship, number: 1141347; Project grant, number: 1122456] and to DKW [Investigator grant, number: 1174040].

**Animal Subjects**

Authors verify that the approval of the institutional animal ethics committee at the University of Melbourne was obtained and experiments performed according to the Australian Guidelines for the Care and Use of Laboratory Animals.

**Conflict of interest**

Authors have no conflict of interest to declare.

**Figure 1. Pediatric TBI results in chronic white matter changes.** a) Whole brain voxel-wise statistical testing revealed a small focus of reduced FA and b) TDI in the ipsilateral corpus callosum. c) Significantly reduced APM extended along the corpus callosum from the ipsilateral to contralateral hemisphere while d) reduced MC was found in deeper structures including the external capsule. The location of each coronal section in a-d is indicated below. All results are fully corrected for FWE and shown overlaid on the inverted study template FA image with significance indicated by the colorbar at top right. TBI = traumatic brain injury; FA = fractional anisotropy; TDI = track density image; APM = average pathlength map; MC = mean curvature; FWE = family-wise error.

**Figure 2. Fixel based analysis revealed significantly reduced fibre density in the ipsilateral corticospinal tract.** Fixels with significantly reduced fibre density (FWE-corrected $p < 0.05$) shown overlaid on the study template FA image with sagittal slice position indicated below. FA = fractional anisotropy; FWE = family-wise error.

**Figure 3.** ***A priori*** **ROI-based analysis of the corpus callosum also revealed significant differences 6 months after pediatric TBI.** Injured mice had a) significantly reduced FA and b) increased RD in the ipsilateral corpus callosum. The track-weighted imaging metrics: c) APM, d) TDI and e) MC were also significantly reduced following injury. ROI = region of interest; TBI = traumatic brain injury; FA = fractional anisotropy; RD = radial diffusivity; TDI = track density image; APM = average pathlength map; MC = mean curvature.

**References**

Anderson, V., Catroppa, C., Morse, S., Haritou, F., & Rosenfeld, J. (2005). Attentional and processing skills following traumatic brain injury in early childhood. *Brain Injury, 19*(9), 699-710.

Anderson, V., & Moore, C. (1995). Age at injury as a predictor of outcome following pediatric head injury: A longitudinal perspective. *Child Neuropsychology, 1*(3), 187-202.

Anderson, V. A., Morse, S. A., Catroppa, C., Haritou, F., & Rosenfeld, J. V. (2004). Thirty month outcome from early childhood head injury: a prospective analysis of neurobehavioural recovery. *Brain, 127*(12), 2608-2620.

Arfanakis, K., Haughton, V. M., Carew, J. D., Rogers, B. P., Dempsey, R. J., & Meyerand, M. E. (2002). Diffusion tensor MR imaging in diffuse axonal injury. *American Journal of Neuroradiology, 23*(5), 794-802.

Armstrong, R. C., Mierzwa, A. J., Marion, C. M., & Sullivan, G. M. (2016). White matter involvement after TBI: Clues to axon and myelin repair capacity. *Experimental Neurology, 275*(3), 328-333.

Assaf, Y., Blumenfeld-Katzir, T., Yovel, Y., & Basser, P. J. (2008). AxCaliber: A method for measuring axon diameter distribution from diffusion MRI. *Magnetic Resonance in Medicine, 59*(6), 1347-1354.

Benson, R. R., Meda, S. A., Vasudevan, S., Kou, Z., Govindarajan, K. A., Hanks, R. A., . . . Coplin, W. (2007). Global white matter analysis of diffusion tensor images is predictive of injury severity in traumatic brain injury. *Journal of neurotrauma, 24*(3), 446-459.

Calamante, F., Tournier, J.-D., Jackson, G. D., & Connelly, A. (2010). Track-density imaging (TDI): super-resolution white matter imaging using whole-brain track-density mapping. *Neuroimage, 53*(4), 1233-1243.

Calamante, F., Tournier, J.-D., Kurniawan, N. D., Yang, Z., Gyengesi, E., Galloway, G. J., . . . Connelly, A. (2012). Super-resolution track-density imaging studies of mouse brain: comparison to histology. *Neuroimage, 59*(1), 286-296.

Calamante, F., Tournier, J.-D., Smith, R. E., & Connelly, A. (2012). A generalised framework for super-resolution track-weighted imaging. *Neuroimage, 59*(3), 2494-2503.

Dhollander, T., Zanin, J., Nayagam, B. A., Rance, G., & Connelly, A. (2018). *Feasibility and benefits of 3-tissue constrained spherical deconvolution for studying the brains of babies.* Paper presented at the Proceedings of the 26th annual meeting of the International Society of Magnetic Resonance in Medicine.

Ewing-Cobbs, L., Prasad, M. R., Swank, P., Kramer, L., Cox Jr, C. S., Fletcher, J. M., . . . Hasan, K. M. (2008). Arrested development and disrupted callosal microstructure following pediatric traumatic brain injury: relation to neurobehavioral outcomes. *Neuroimage, 42*(4), 1305-1315.

Faul, M., Xu, L., Wald, M., Coronado, V., & Dellinger, A. M. (2010). Traumatic brain injury in the United States: national estimates of prevalence and incidence, 2002–2006. *Injury Prevention, 16*(Suppl 1), A268-A268.

Flygt, J., Djupsjö, A., Lenne, F., & Marklund, N. (2013). Myelin loss and oligodendrocyte pathology in white matter tracts following traumatic brain injury in the rat. *European Journal of Neuroscience, 38*(1), 2153-2165.

Frank, L. R. (2002). Characterization of anisotropy in high angular resolution diffusion- weighted MRI. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine, 47*(6), 1083-1099.

Genc, S., Anderson, V., Ryan, N. P., Malpas, C. B., Catroppa, C., Beauchamp, M. H., & Silk, T. J. (2017). Recovery of white matter following pediatric traumatic brain injury depends on injury severity. *Journal of neurotrauma, 34*(4), 798-806.

Giza, C. C., & Prins, M. L. (2006). Is being plastic fantastic? Mechanisms of altered plasticity after developmental traumatic brain injury. *Developmental neuroscience, 28*(4-5), 364-379.

Harris, N. G., Verley, D., Gutman, B. A., & Sutton, R. (2016). Bi-directional changes in fractional anisotropy after experiment TBI: disorganization and reorganization? *Neuroimage, 133*, 129-143.

Huisman, T. A., Schwamm, L. H., Schaefer, P. W., Koroshetz, W. J., Shetty-Alva, N., Ozsunar, Y., . . . Sorensen, A. G. (2004). Diffusion tensor imaging as potential biomarker of white matter injury in diffuse axonal injury. *American Journal of Neuroradiology, 25*(3), 370-376.

Jensen, J. H., Helpern, J. A., & Tabesh, A. (2014). Leading non-Gaussian corrections for diffusion orientation distribution function. *NMR in Biomedicine, 27*(2), 202-211.

Kamper, J. E., Pop, V., Fukuda, A. M., Ajao, D. O., Hartman, R. E., & Badaut, J. (2013). Juvenile traumatic brain injury evolves into a chronic brain disorder: behavioral and histological changes over 6 months. *Exp Neurol, 250*, 8-19.

Kumar, R., Husain, M., Gupta, R. K., Hasan, K. M., Haris, M., Agarwal, A. K., . . . Narayana, P. A. (2009). Serial changes in the white matter diffusion tensor imaging metrics in moderate traumatic brain injury and correlation with neuro-cognitive function. *Journal of neurotrauma, 26*(4), 481-495.

Lazar, M., Jensen, J. H., Xuan, L., & Helpern, J. A. (2008). Estimation of the orientation distribution function from diffusional kurtosis imaging. *Magnetic Resonance in Medicine, 60*(4), 774-781.

Levin, H. S., Benavidez, D., Verger-Maestre, K., Perachio, N., Song, J., Mendelsohn, D., & Fletcher, J. (2000). Reduction of corpus callosum growth after severe traumatic brain injury in children. *Neurology, 54*(3), 647-647.

Lu, H., Jensen, J. H., Ramani, A., & Helpern, J. A. (2006). Three-dimensional characterization of non-gaussian water diffusion in humans using diffusion kurtosis imaging. *NMR in Biomedicine*, *19*, 236-247.

Mac Donald, C. L., Dikranian, K., Bayly, P., Holtzman, D., & Brody, D. (2007). Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. *Journal of Neuroscience, 27*(44), 11869-11876.

Malhotra, A., Sepehrizadeh, T., Dhollander, T., Wright, D., Castillo-Melendez, M., Sutherland, A. E., … Miller S. L. (2019) Advanced MRI analysis to detect white matter brain injury in growth restricted newborn lambs. *NeuroImage: Clinical, 24*, 101991.

Merkley, T. L., Bigler, E. D., Wilde, E. A., McCauley, S. R., Hunter, J. V., & Levin, H. S. (2008). Diffuse changes in cortical thickness in pediatric moderate-to-severe traumatic brain injury. *Journal of neurotrauma, 25*(11), 1343-1345.

Newcombe, V., Williams, G., Nortje, J., Bradley, P., Harding, S., Smielewski, P., . . . Hutchinson, P. (2007). Analysis of acute traumatic axonal injury using diffusion tensor imaging. *British journal of neurosurgery, 21*(4), 340-348. Retrieved from https://www.tandfonline.com/doi/full/10.1080/02688690701400882

Norenberg, M. D., Smith, J., & Marcillo, A. (2004). The pathology of human spinal cord injury: defining the problems. In: Mary Ann Liebert, Inc.

Palacios, E. M., Fernandez-Espejo, D., Junque, C., Sanchez-Carrion, R., Roig, T., Tormos, J. M., . . . Vendrell, P. (2011). Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury. *BMC neurology, 11*(1), 24.

Pannek, K., Mathias, J. L., Bigler, E. D., Brown, G., Taylor, J. D., & Rose, S. E. (2011). The average pathlength map: a diffusion MRI tractography-derived index for studying brain pathology. *Neuroimage, 55*(1), 133-141.

Potts, M. B., Koh, S.-E., Whetstone, W. D., Walker, B. A., Yoneyama, T., Claus, C. P., . . . Noble-Haeusslein, L. J. (2006). Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. *NeuroRx, 3*(2), 143-153.

Pullela, R., Raber, J., Pfankuch, T., Ferriero, D. M., Claus, C. P., Koh, S.-E., . . . Noble-Haeusslein, L. J. (2006). Traumatic injury to the immature brain results in progressive neuronal loss, hyperactivity and delayed cognitive impairments. *Developmental neuroscience, 28*(4-5), 396-409.

Raffelt, D. A., Smith, R. E., Ridgway, G. R., Tournier, J.-D., Vaughan, D. N., Rose, S., . . . Connelly, A. (2015). Connectivity-based fixel enhancement: Whole-brain statistical analysis of diffusion MRI measures in the presence of crossing fibres. *Neuroimage, 117*, 40-55.

Raffelt, D. A., Tournier, J.-D., Smith, R. E., Vaughan, D. N., Jackson, G., Ridgway, G. R., & Connelly, A. (2017). Investigating white matter fibre density and morphology using fixel-based analysis. *Neuroimage, 144*, 58-73.

Robinson, S., Winer, J. L., Berkner, J., Chan, L. A., Denson, J. L., Maxwell, J. R., . . . Meehan, W. P. (2016). Imaging and serum biomarkers reflecting the functional efficacy of extended erythropoietin treatment in rats following infantile traumatic brain injury. *Journal of Neurosurgery: Pediatrics, 17*(6), 739-755.

Ryan, N. P., Anderson, V., Godfrey, C., Beauchamp, M. H., Coleman, L., Eren, S., . . . Catroppa, C. (2014). Predictors of very-long-term sociocognitive function after pediatric traumatic brain injury: evidence for the vulnerability of the immature "social brain". *Journal of neurotrauma, 31*(7), 649-657.

Ryan, N. P., van Bijnen, L., Catroppa, C., Beauchamp, M. H., Crossley, L., Hearps, S., & Anderson, V. (2016). Longitudinal outcome and recovery of social problems after pediatric traumatic brain injury (TBI): Contribution of brain insult and family environment. *International journal of developmental neuroscience, 49*, 23-30.

Semple, B. D., O'Brien, T. J., Gimlin, K., Wright, D. K., Kim, S. E., Casillas-Espinosa, P. M., . . . Noble-Haeusslein, L. J. (2017). Interleukin-1 Receptor in Seizure Susceptibility after Traumatic Injury to the Pediatric Brain. *J Neurosci, 37*(33), 7864-7877. doi:10.1523/jneurosci.0982-17.2017

Semple, B. D., Trivedi, A., Gimlin, K., & Noble-Haeusslein, L. J. (2015). Neutrophil elastase mediates acute pathogenesis and is a determinant of long-term behavioral recovery after traumatic injury to the immature brain. *Neurobiology of disease, 74*, 263-280.

Smith, S. M., & Nichols, T. E. (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage, 44*(1), 83-98.

Song, S.-K., Sun, S.-W., Ju, W.-K., Lin, S.-J., Cross, A. H., & Neufeld, A. H. (2003). Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage, 20*(3), 1714-1722.

Suskauer, S. J., & Huisman, T. A. (2009). Neuroimaging in pediatric traumatic brain injury: current and future predictors of functional outcome. *Developmental disabilities research reviews, 15*(2), 117-123.

Tournier J.-D., Mori, S., & Leemans, A. (2011). Diffusion tensor imaging and beyond. *Magnetic Resonance in Medicine, 65*(6),1532-1556.

Wilde, E. A., Ayoub, K. W., Bigler, E. D., Chu, Z. D., Hunter, J. V., Wu, T. C., . . . Levin, H. S. (2012). Diffusion tensor imaging in moderate-to-severe pediatric traumatic brain injury: changes within an 18 month post-injury interval. *Brain imaging and Behavior, 6*(3), 404-416.

Wilde, E. A., Bigler, E. D., Hunter, J. V., Fearing, M. A., Scheibel, R. S., Newsome, M. R., . . . Levin, H. S. (2007). Hippocampus, amygdala, and basal ganglia morphometrics in children after moderate- to- severe traumatic brain injury. *Developmental Medicine & Child Neurology, 49*(4), 294-299.

Wortman, R. C., Meconi, A., Neale, K. J., Brady, R. D., McDonald, S. J., Christie, B. R., . . . Shultz, S. R. (2018). Diffusion MRI abnormalities in adolescent rats given repeated mild traumatic brain injury. *Ann Clin Transl Neurol, 5*(12), 1588-1598. doi:10.1002/acn3.667

Wozniak, J. R., Krach, L., Ward, E., Mueller, B. A., Muetzel, R., Schnoebelen, S., . . . Lim, K. O. (2007). Neurocognitive and neuroimaging correlates of pediatric traumatic brain injury: a diffusion tensor imaging (DTI) study. *Archives of Clinical Neuropsychology, 22*(5), 555-568.

Wright, D. K., Brady, R. D., Kamnaksh, A., Trezise, J., Sun, M., McDonald, S. J., . . . Johnston, L. A. (2019). Repeated mild traumatic brain injuries induce persistent changes in plasma protein and magnetic resonance imaging biomarkers in the rat. *Scientific reports, 9*(1), 1-13.

Wright, D. K., Johnston, L. A., Kershaw, J., Ordidge, R., O'Brien, T. J., & Shultz, S. R. (2017). Changes in apparent fiber density and track-weighted imaging metrics in

white matter following experimental traumatic brain injury. *Journal of Neurotrauma, 34*(13), 2109-2118.

Wright, D. K., Liu, S., Van Der Poel, C., McDonald, S. J., Brady, R. D., Taylor, L., . . . O'Brien, T. J. (2016). Traumatic brain injury results in cellular, structural and functional changes resembling motor neuron disease. *Cerebral cortex, 27*(9), 4503-4515.

Wright, D. K., Liu, S., Van Der Poel, C., McDonald, S. J., Brady, R. D., Taylor, L., . . . O'Brien, T. J. (2017). Traumatic brain injury results in cellular, structural and functional changes resembling motor neuron disease. *Cerebral cortex, 27*(9), 4503-4515.

Wright, D. K., O'Brien, T. J., Shultz, S. R., & Mychasiuk, R. (2017). Sex matters: repetitive mild traumatic brain injury in adolescent rats. *Annals of clinical and translational neurology, 4*(9), 640-654.

Wright, D. K., Trezise, J., Kamnaksh, A., Bekdash, R., Johnston, L. A., Ordidge, R., . . . O'Brien, T. J. (2016). Behavioral, blood, and magnetic resonance imaging biomarkers of experimental mild traumatic brain injury. *Scientific reports, 6*, 28713.

Zhang, H., Schneider, T., Wheeler-Kingshott, C. A., & Alexander, D. C. (2012). NODDI: Practical *in vivo* neurite orientation dispersion and density imaging of the human brain. *NeuroImage, 61*(4), 1000-1016.

Figure 1:

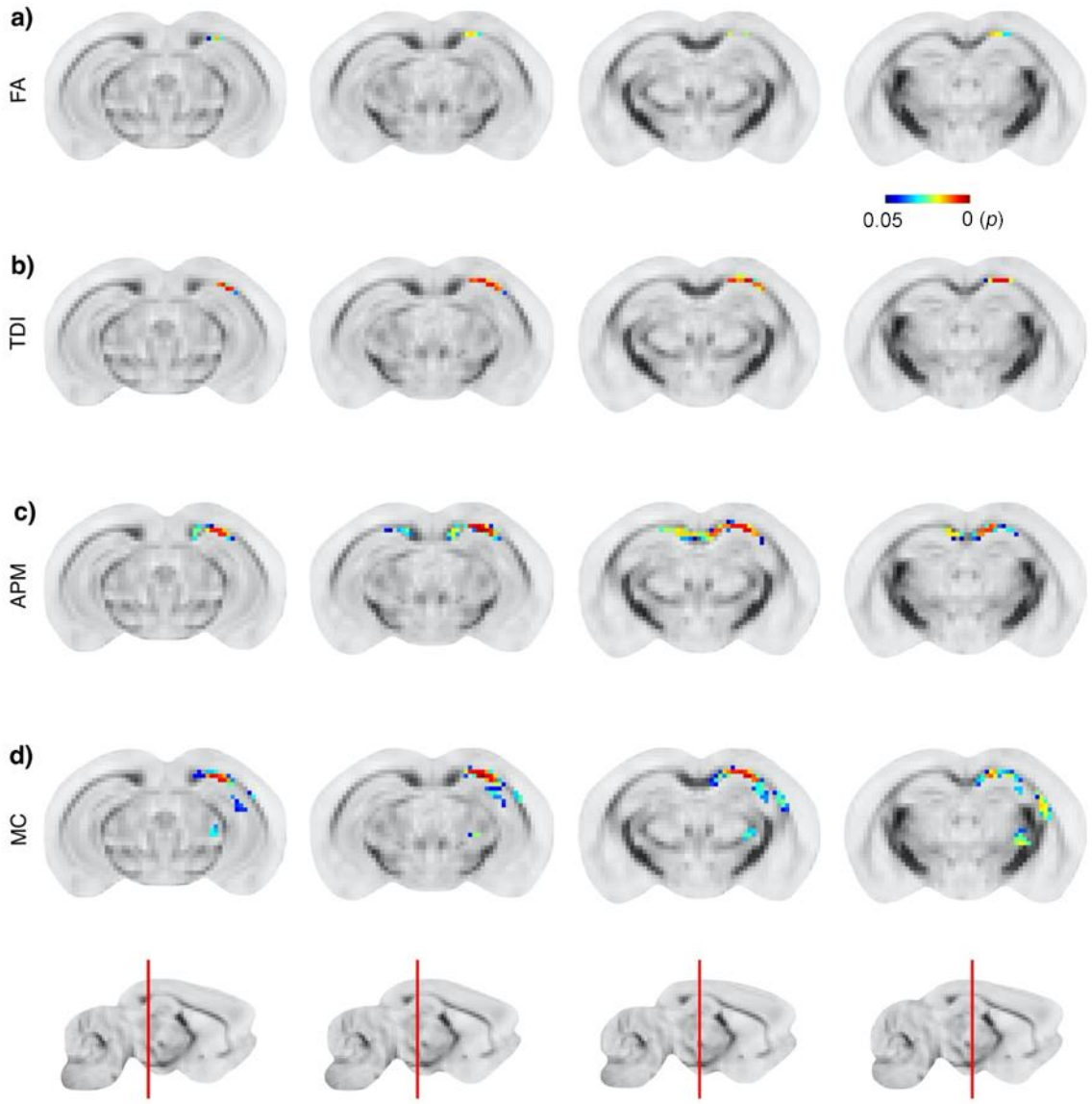


Figure 2:

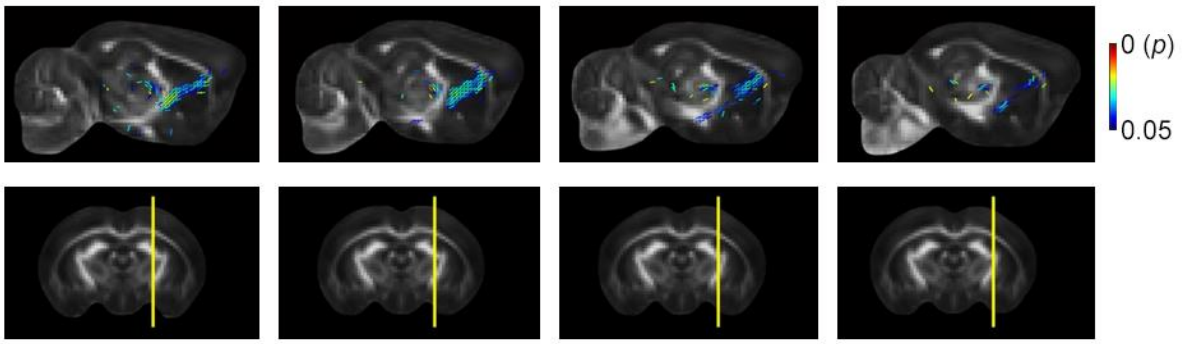
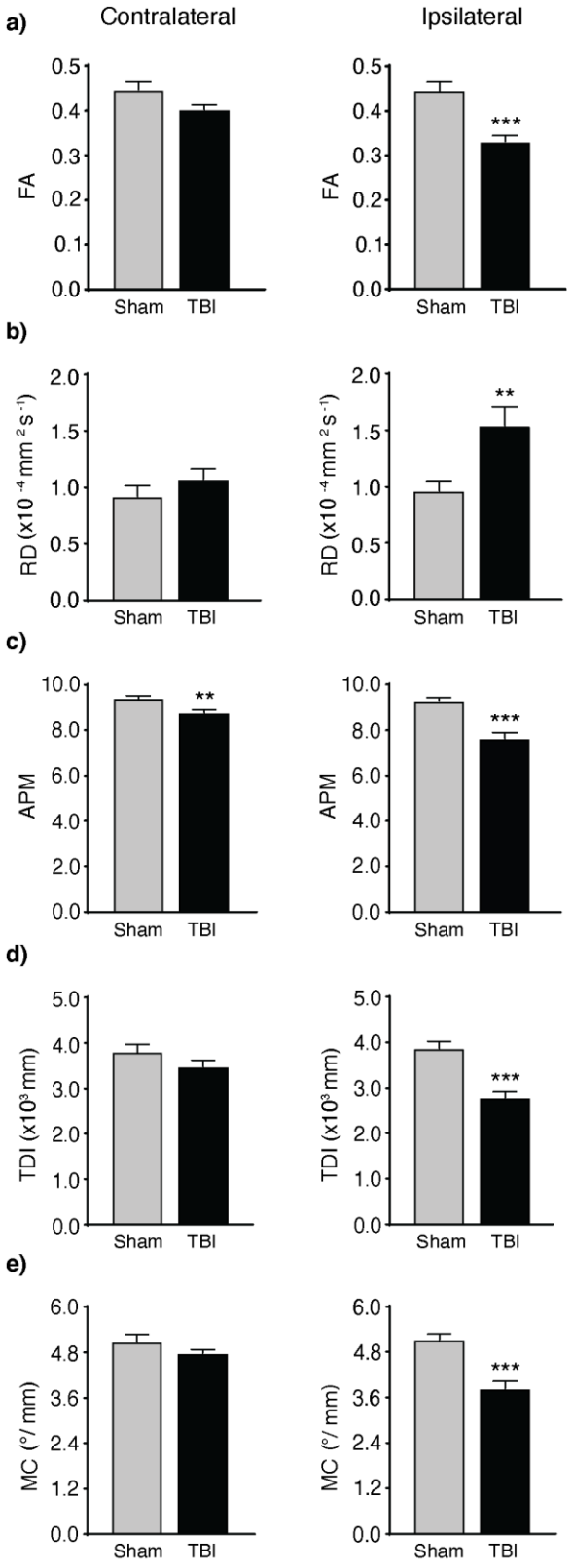


Figure 3:


a)
Contralateral
Ipsilateral
FA
0.5
0.4
0.3
0.2
0.1
0.0
***
Sham
TBI
b)
RD (x10 -4 mm 2 s -1)
2.0
1.5
1.0
0.5
0.0
**
c)
APM
10.0
8.0
6.0
4.0
2.0
d)
TDI (x10³ mm)
5.0
4.0
3.0
2.0
1.0
e)
MC (°/ mm)
6.0
4.8
3.6
2.4
1.2