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# Bi-directional changes in fractional anisotropy after experiment TBI: Disorganization and reorganization?

N.G. Harris [a,*], D.R. Verley [a], B.A. Gutman [b], R.L. Sutton [a]

[a] UCLA Brain injury Research Center, Department of Neurosurgery, University of California, Los Angeles, Los Angeles, USA
[b] Department of Neurology, Imaging Genetics Center, Keck/USC School of Medicine, Institute for Neuroimaging and Informatics, University of Southern California, Los Angeles, CA, USA

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ABSTRACT

The current dogma to explain the extent of injury-related changes following rodent controlled cortical impact (CCI) injury is a focal injury with limited axonal pathology. However, there is in fact good, published histologic evidence to suggest that axonal injury is far more widespread in this model than generally thought. One possibility that might help to explain this is the often-used region-of-interest data analysis approach taken by experimental traumatic brain injury (TBI) diffusion tensor imaging (DTI) or histologic studies that might miss more widespread damage, when compared to the whole brain, statistically robust method of tract-based analysis used more routinely in clinical research. To determine the extent of DTI changes in this model, we acquired *in vivo* DTI data before and at 1 and 4 weeks after CCI injury in 17 adult male rats and analyzed parametric maps of fractional anisotropy (FA), axial, radial, and mean diffusivity (AD, RD, MD), tensor mode (MO), and fiber tract density (FTD) using tract-based spatial statistics. Contusion volume was used as a surrogate marker of injury severity and as a covariate for investigating severity dependence of the data. Mean fiber tract length was also computed from seeds in the cortical spinal tract regions. In parallel experiments ($n = 3$–5/group), we investigated corpus callosum neurofilaments and demyelination using immunohistochemistry (IHC) at 3 days and 6 weeks, callosal tract patency using dual-label retrograde tract tracing at 5 weeks, and the contribution of gliosis to DTI parameter maps using GFAP IHC at 4 weeks post-injury. The data show widespread ipsilateral regions of significantly reduced FA at 1 week post-injury, driven by temporally changing values of AD, RD, and MD that persist to 4 weeks. Demyelination, retrograde label tract loss, and reductions in MO (tract degeneration) and FTD were shown to underpin these data. Significant FA increases occurred in subcortical and corticospinal tract regions that were spatially distinct from regions of FA decrease, grossly affected gliotic areas, and MO changes. However, there was good spatial correspondence between regions of increased FA and areas of increased FTD and mean fiber length. We discuss these widespread changes in DTI parameters in terms of axonal degeneration and potential reorganization, with reference to a resting state fMRI companion paper (Harris et al., 2016, Exp. Neurol. 227:124–138) that demonstrated altered functional connectivity data acquired from the same rats used in this study.



## Introduction

Over the past two decades or more, diffusion tensor imaging (DTI) has gained wide acceptance for delineating white matter pathology after traumatic brain injury (TBI). It is being used increasingly for clinical research-based detection of abnormalities in mild to severe TBI patients (Rutgers et al., 2008) including blast injury (Mac Donald et al., 2011). The commonly used diffusion indices derived from the tensor model fit of DTI data provide insight into the directionality and magnitude of the movement of protons in the presence of diffusion-sensitizing gradients. A full understanding of the tissue pathology changes that result in alteration in diffusion characteristics is an ongoing process in many CNS diseases states, for example, multiple sclerosis (Budde et al., 2009; Chiang et al., 2014; Song et al., 2002) as well as TBI (Budde et al., 2011; Laitinen et al., 2010, 2015; MacDonald et al., 2007). Given the potential importance of DTI metrics as biomarkers of disease stage, severity, and outcome, a thorough study relating pathogenesis and imaging parameters is required to promote their understanding and wider use. In addition to using fractional anisotropy (FA), the degree of unidirectional movement of protons within a gradient, as an indicator of axonal integrity after TBI, it is also used to delineate fiber tract pathways using one of the many tracking algorithms available. As a result, it is important to determine when DTI metrics become less affected by altered tissue integrity post-injury, for example, vasogenic edema or increased cell density (Chiang et al., 2014). This

* Corresponding author at: Department of Neurosurgery, David Geffen School of Medicine, UCLA, 300 Stein Plaza, Ste 535, Box 956901, Los Angeles, CA 90095-7039. Fax: +1 801 672 1018.
*E-mail address:* ngharris@mednet.ucla.edu (N.G. Harris).

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should increase confidence that FA and tractography data used in the computation of structural connectomes can be relied upon to reflect solely a measure of patent fiber tract pathways and network changes and is free from confounds related to continuing disease pathology.

While evidence from adult rodent experimental TBI studies has shown that DTI parameters closely relate to axonal injury within 24 h of a cortical contusion in mice (MacDonald et al., 2007), this relationship has been found to be substantially influenced by gliosis in gray matter at 2 months post-injury in a rat contusion model (Budde et al., 2011). These and other studies in rodent TBI models (Laitinen et al., 2015; Zhuo et al., 2012) indicate that the interpretation of pathology corresponding to DTI parameter changes is not straightforward and that further study is required. Perhaps even more controversial in the TBI field is the finding and interpretation of an increase of FA after injury. There are several clinical investigations that report this and discuss the implications in terms of pathology, injury severity, and functional outcome within regions of both white and gray matter (Cubon et al., 2011; Ling et al., 2012; Lipton et al., 2012; Mayer et al., 2015; Messé et al., 2010). The majority of studies using *in vivo* DTI in adult rodent TBI models report decrease in white matter FA in association with axonal injury (Laitinen et al., 2015; Long et al., 2015; MacDonald et al., 2007; Sierra et al., 2011; Xu et al., 2011). Most experimental DTI studies use a region-of-interest (ROI)-based approach to data analyses that might miss some important changes related to more subtle injury pathology. In fact, of the few existing studies using whole brain, voxel-based analysis of DTI parameters, an increase rather than a decrease in thalamic FA was reported at 30 days after blast injury in the mouse (Rubovitch et al., 2011) as well as in the ipsilateral barrel cortex at 12 weeks after lateral fluid percussion injury (Johnstone et al., 2015). Therefore, it remains a possibility that a more statistically robust, whole brain analysis of white matter tracts will provide a greater indication of pathology after TBI in rodent models. As whole brain analysis is used routinely in clinical studies, such a study may also uncover greater similarities to clinical data. In the controlled cortical impact injury (CCI) model of TBI, contrary to current dogma on the focal nature of the injury, there is in fact widespread axonal degeneration both bilaterally as well as caudally in the forebrain after CCI injury, as indicated by silver staining data (Hall et al., 2005, 2008; Matthews et al., 1998). We hypothesize that a whole brain approach to rodent DTI analysis would yield a better approximation to the total amount of axonal damage present after CCI injury as reflected in those studies.

Based on these prior data and preliminary work using DTI in this model (Harris et al., 2009), we acquired *in vivo* DTI data before and at 1 and 4 weeks after injury using the CCI injury model in adult rats, in an effort to understand the temporal and spatial nature of FA changes after TBI. We hypothesized that a white matter tract-based spatial statistical approach to rodent DTI analysis would provide a more widespread delineation of axonal pathology after rodent contusion injury more comparable to prior silver staining studies. We also acquired dual-label, retrograde dye-injection tract tracing data together with immunohistochemistry data of myelination and gliosis status to provide further verification of the DTI changes. We discuss these data in terms of the many known confounds that underlie the DTI indices, and with particular reference to a resting state fMRI companion paper (Harris et al., 2016) that demonstrated significantly altered functional connectivity from data that we acquired from the same rats and similar time points used in this study.

## Methods

### *Experiment protocol*

DTI data were acquired on a 7-T Bruker MRI from adult rats ($n = 17$) under isofluorane sedation before CCI injury and at 7 and 28 days after brain injury. Additional groups of rats were used for dual-label tract tracing at 35 days post-injury ($n = 3$/group) and for immunohistology at 3, 28, and 42 days post-injury ($n = 3$–5/group).

### *Brain injury*

All study protocols were approved by the University of California Los Angeles Chancellor's Animal Research Committee and adhered to the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The method for induction of moderate CCI injury was performed in the manner similar to previously described.(Chen et al., 2002, 2003, 2004; Harris et al., 2010a, 2010b, 2012) Briefly, male, Sprague–Dawley rats (220–250 g body weight) were anaesthetised with 2% isofluorane vaporized in $O_2$ flowing at 0.6 L/min and placed on a homeostatic temperature-controlled blanket while maintained in a stereotactic frame. CCI injury was produced using a 4-mm diameter impactor tip that was advanced through a 5-mm craniotomy (centered at 0.0 mm Bregma and 3-mm left lateral to the sagittal suture) onto the brain using a 20-psi pressure pulse, and to a deformation depth of 2 mm below the dura. The craniotomy site was covered with a non-toxic, rapid curing silicone elastomer (WP Instruments, USA), and the wound was closed with sutures.

### *MRI acquisition*

Rats were briefly anesthetized with 4% isofluorane in oxygen flowing at 0.6 l/min and then transferred to a purpose-built cradle and secured using three-point immobilization of the head with two ear bars and a tooth bar. Resting state functional MRI (rsFMRI) data were then acquired from all rats under medetomidine sedation for 45 min prior to resuming isoflurane sedation at 1.2–1.5% for the remainder of the imaging session to collect the diffusion imaging data reported in this manuscript. This rsFMRI data and the details of the medetomidine sedation has been published (Harris et al., 2016). The rat cradle was placed in the center of a 7-T spectrometer (Oxford Instr, Carteret, NJ, USA) driven by a Bruker console running Paravision 5.1 (Billerica, MA USA). Respiration was monitored remotely and temperature was homeothermically controlled by forced air (SA11 Instr, Inc., USA). The S116 Bruker gradients (400 mT/m) were used in combination with a birdcage transmit and an actively decoupled, receive-only surface coil to acquire the data. Following a multi-slice gradient-echo pilot scan to optimize positioning within the magnet, localized shimming was performed on the head to improve B0 homogeneity. A standard, 4-shot, spin echo, echo planar imaging sequence (6250/32 ms repetition and echo time, respectively) was used to acquire diffusion-weighted images with directionally encoded gradients applied along 30 different, even-spaced directions and with a $b$ value of 1000 s/mm$^2$, using $\Delta = 20$ ms and $\delta = 3$ ms. Five additional images were acquired with a $b$ value of 0 s/mm$^2$. All images were acquired with a 128-read and 128-phase-encoding matrix ($X$,$Y$ direction respectively) within a 35-mm$^2$ field-of-view and 25 × 0.75 mm contiguous, coronal slices, resulting in an in-plane resolution of 273 × 273 μm and a 750-μm slice thickness. Anatomical, T2-weighted, rapid-relaxation-with-enhancement (RARE) data were acquired with 50 × 0.5 mm slices, 128-read × 128-phase-encoding matrix within a 35 × 35 mm field-of-view, a TR/TE 5000/60 ms repetition and echo time, respectively, a RARE factor of 8 and 2 averages.

### *Retrograde Tract Tracing and Immunostaining*

Additional injured (post-injury day 28) and naïve control rats that were not imaged in this study ($n = 3$/group) were used for tract tracing. The retrograde tract tracers Fluorogold (FG, 5% solution Sigma-Alrich, USA) in artificial cerebrospinal fluid (aCSF, Harvard Apparatus, USA) and cholera toxin B (CTB, 1% solution in aCSF, List Biological Labs, Campbell, CA, USA) were injected into layer V of the cortex (1.5 mm below dura) lateral (0 mm, Bregma, 5 mm lateral) and medial (0 mm Bregma, 0.5 mm lateral) to the contusion, respectively, using 1.5 μl of each