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Diffusion imaging of mild traumatic brain injury in the impact accelerated rodent model: a pilot study

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Abstract

Primary Objective—There is a need to understand brain pathologic processes following mild traumatic brain injury (mTBI). Previous diffusion MRI studies report axonal injury and edema in the first week after injury in a rodent model. This study aims to investigate the processes occurring one week after injury at time of regeneration and degeneration using dMRI in the impact acceleration rat mTBI model.

Research Design—Eighteen rats were subjected to impact acceleration injury, and three rats served as sham controls. Seven days post-injury, dMRI was acquired from fixed rat brains using a 7T scanner. Group comparison of Fractional Anisotropy (FA) values between traumatized and sham animals was performed using Tract-Based Spatial Statistics (TBSS), a method that we adapted for rats.

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Declaration of interest

The authors report no conflict of interest. This work was supported, in part, by Center for Integration of Medicine and Innovative Technology (CIMIT)(Award title: Improving Imaging of Diffuse Axonal Injury in Traumatic Brain Injury)(MES, BK); Memorial Award, Else Kröner-Fresenius Foundation, Germany (IK)

Main Outcomes and Results—TBSS revealed brain white matter regions with increased FA values in the traumatized versus sham rats, localized mainly to the contrecoup region. Regions of increased FA included fiber tracts of the corpus callosum, the anterior commissure, the cerebellar peduncles, the fimbria of the hippocampus, the fornix, the medial forebrain bundle, the optic chiasm, the pyramidal tract, and the spinal trigeminal tract.

Conclusion—Seven days post-injury, during the period of tissue reparation in the impact acceleration rat model of mTBI, microstructural changes to white matter can be detected using dMRI.

Keywords

Animal models; Traumatic Brain Injury (TBI); Neuroimaging; Closed head injury

Introduction

There is a need to understand processes occurring as a consequence of human traumatic brain injury (TBI). Every year, traumatic brain injuries lead to a considerable number of cases of emergency room visits (1.4 million visits) in the UK[1]. A high percentage of these injuries, about 75%, are concussions, also known as mild traumatic brain injury (mTBI)[2]. Mild TBI is a consequence of an injury to the head and can occur in multiple situations, including daily life (e.g., falls, automotive accidents), in sports (e.g., soccer, rugby), or in military circumstances, (e.g., improvised explosive devices)[3]. An increasing number of mTBI cases are occurring and are being diagnosed in the UK[4–6] and in the USA in military personnel[7] deployed to Iraq and Afghanistan. There is a clinical need to improve the assessment, diagnosis, and long-term outcome of mTBI for both military and civilian populations. Clinically, neuropsychological testing, conventional computerized tomography (CT), and conventional magnetic resonance imaging (MRI), are recognized to have only limited sensitivity to detect and to characterize the injuries observed in mTBI. Injuries from mTBI are typically associated with diffuse axonal injury, which are not detected using conventional imaging methods. As a result of a lack of sufficiently sensitive diagnostic methods, brain injury in mTBI is being underestimated and poorly treated[8–10](e.g., see also review in [11]).

In contrast to CT and structural MRI, diffusion tensor imaging (dMRI) is an imaging technique that is sensitive to detecting subtle, diffuse white matter changes in the brain. dMRI is a relatively new method and especially promising as it is a non-invasive *in vivo* imaging technique that can be used in most clinical settings. dMRI is a technique that is based on the phenomenon of water diffusion in tissue[12,13] and it is used to detect changes in the structure, organization, and directionality of gray matter and white matter fiber bundles in the brain. A common output measure of dMRI is the Fractional Anisotropy (FA). FA describes the degree of anisotropy of water diffusion in tissue, with values ranging between 0 and 1, and has higher values in regions of white matter with well-organized, myelinated fiber tracts. dMRI is a method of choice to detect subtle changes in brain white matter tissue.

In order to advance the understanding of mTBI, there is a need to advance animal models where specific scenarios of the injury and their relation to imaging findings can be explored under controlled laboratory settings. Animal studies that report the effects of mild brain injury with respect to behavioural, vascular, histological, and hormonal changes are numerous (summarized e.g.[14,15]), but only a handful of studies included brain imaging. One of the established models of mTBI is the impact acceleration model [16–18]. This rat model has been explored during the first few hours to days of injury reporting the presence of axonal injury and edema [19–22]. However, what has not yet been investigated using dMRI is the period of regeneration and degeneration that begins one week after injury.

In this study, we subjected rats to an impact acceleration brain injury and evaluated brain white matter microstructure using dMRI 7 days post-injury. The aim of this study was to compare the white matter microstructure in the injured versus the non-injured animals.

Methods

Animals and experimental design

Adult male rats, (n=21), age 7 weeks, weight 330g, Sprague-Dawley strain, were used. The protocol of the impact acceleration rat model of injury was followed[16,18]. Each of the 21 animals had a stainless steel disk, 10 mm in diameter and 3 mm in thickness, glued to the surface of the left parietal bone, centered between the bregma and lambda. The animal was placed on a 10 cm thick foam bed that was padded either with a soft (Foam Type 1010, foam stiffness characterized in Indention Force Deflection (IFD) = 10 => 10 pounds pressure to compress 25%), medium, (Foam type 1845, ie, IFD => 45 pounds pressure to compress 25%) or firm foam (IFD>60). The impact was delivered on this disk with a weight of 300g dropped from a height of 129 cm, 137.5 cm or 147.5 cm. The rat was removed from the device after a single impact, the disk removed, and the incision closed. The control animals (n=3) were subjected to all procedures excluding impact. All animals were sacrificed one week post-injury. The animal protocol was approved by Harvard Medical School Institutional Animal Care and Use Committee (IACUC) and the Animal Care and Use Review Office (ACURO) of the US Army.

Tissue fixation for dMRI—On day 7, rats were anesthetized, perfused, and the brains fixed within the skull using formaldehyde. Thereafter, the bulk of the soft tissue was stripped, leaving just the brain and calvarium, and the sample was washed 3 times in PBS solution with 0.05% sodium azide over the course of one week. Just prior to MRI scanning the samples were placed in Dupont Krytox 1506 fluid to eliminate signal outside the sample.

Image acquisition—Fixed rat brains were scanned on a 7T-MRI system Biospec 70/20 USR (Bruker BioSpin MRI GmbH, Ettlingen, Germany) at Northeastern University (NEU), Boston, USA. High spatial resolution and high signal-to-noise ratio for diffusion weighted images (dMRI) was achieved using the following methods. First, segmented 3D echo-planar spin-echo diffusion imaging datasets were obtained for each animal brain, with k-space divided into 8 sections. One baseline, non-diffusion weighted scan and 12 isotropically oriented diffusion gradient directions were acquired. Other parameters included: b-value=800 s/mm2; δ (diff gradient length)=4ms; Δ (diff gradient separation)=8ms; TE

19ms; resolution 250μm isotropic (matrix 128×96×96); TR=500ms;1 average. Scan time was one hour and 23 minutes.

DMRI data analysis—Group comparison between the injured and the sham animals was carried out using the Tract-Based Spatial Statistics (TBSS) method[23], which is part of FMRIB Software Library (FSL) [24]. We have chosen this method because it allows the comparison of the whole FA image voxel by voxel across animals. This was achieved, first, by a registration of the FA images of all animals to a selected image (the target image), second, by generating a 'skeleton', which represents all tracts that are common in all images and represents each such tract as a single line running through the center of the tract, and third, by carrying out a comparison for each voxel on the skeleton across animals and visualizing statistically significant group differences for maps at the p value of 0.05 without correction for Family Wise Error (FWE). The original TBSS method is optimized for the human brain, and here we adjusted the template to the rat brain. Modifications to TBSS are described below, but, when applicable, the steps described in the TBSS user guide were followed (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS).

In short, scalar FA images were created from diffusion-weighted images (DWI) using internal laboratory scripts. Brain masks were generated from the DWI using threshold estimation in 3DSlicer software version 4 (http://www.slicer.org)[25]. All 3D rat images were submitted to nonlinear registration and the best target image was selected, following TBSS user guidelines. The next step involved bringing the rat images into standard space and merging them into one single 4D image, which was then modified by using a script in which each image was aligned to the space of the rat target image instead of to the MNI152 space used in humans. Following this, a skeleton was created according to the TBSS protocol and thresholded at 0.3. Each animal's aligned FA data was then projected onto this skeleton and the resulting data was then used in voxelwise cross-subject statistics to obtain regions of difference in FA between the control and the injured animals. FA was quantified from each animal as directed in the TBSS guide: the 4D image was split into individual images and FA was extracted. We extracted both the average FA over the entire skeleton (figure 1) as well as the FA over the regions where voxel-wise comparison had shown significant differences (figures 2 and 3). The image used as the target image was not used in the final TBSS output and therefore we present results from 17 injured animals and the three controls.

Statistical Analysis—The Mann-Whitney and the Kruskal-Wallis non-parametric tests were used to investigate changes in FA between the traumatized and control animals. This was followed up with a post-hoc Tukey test to control for multiple t-comparisons. We used SPSS version 22.0.0 (IBM software) to calculate the statistics.

Results

DMRI image analysis of white matter

Eighteen rats were subjected to impact acceleration injury and three rats served as controls. To test whether any measurable changes to the white matter occurred in response to injury, major white matter tracts of the whole brain were extracted using the TBSS method, and FA was compared between the injured animals and the control group. The average FA extracted

from the entire skeleton was statistically significantly higher in the injured rat group than in the non-injured group (Mann-Whitney test, $U_{(19)}$=6, Z= −2.064, p = 0.039) (see figure 1).

To determine whether or not changes in FA were spread over the entire white matter in the brain or whether they were localized to specific areas, we followed up with voxel-wise statistics of the white matter skeleton using the TBSS method. This analysis revealed specific regions with increased FA values in the traumatized rats ($p<0.05$ without correcting for family-wise errors) (figure 2). FA extracted from these specific regions was significantly different between the injured and the non-injured group (Mann-Whitney U = 0, Z=−2.7, $p_{(2\ tailed)}$ = 0.007). Specifically, FA was higher in the injured animals (mean FA of 0.41 SD 0.01) than in the control animals (mean FA of 0.37 SD 0.01) (see figure 3). The regions of increased FA were localized to the intrabulbar portion of the anterior commissure, the cerebellar peduncles (the inferior and the superior), the forceps minor of the corpus callosum, the fimbria of the hippocampus, the fornix, the medial forebrain bundle, the optic chiasm, the pyramidal tract, and the spinal trigeminal tract (see figure 2). In summary, the pattern of the lesions was concentrated in the ventral regions of the rat brain and the brain stem, corresponding to the contrecoup region. Interestingly, there were no changes in FA in the region of the primary impact, the coup region.

Height and foam firmness as factor of the severity of injury

To test experimental parameters of the impact, three foam types of different firmness (soft, medium and firm), and three different heights of drops (129 cm, 137.5 cm or 147.5 cm) were used. The rationale here was that dropping weights from higher heights would create more extensive damage than lower heights, and that decreased firmness of the bed padding would result in more extensive damage to the long white matter fiber tracts. The injured animals were divided in subgroups according to the height from which the impact was administered (129 cm, 137.5 cm, respectively 147.5 cm) and average FA over the contrecoup regions was compared. We performed a Kruskall-Wallis test, a non-parametric test, to detect possible differences between these three experimental conditions (height 129 cm, 137.5 cm, 147.5 cm). There were no significant group differences observed, suggesting that there were no differences among the three drop heights. In a second analysis, the injured animals were divided in subgroups according to the firmness of the foam padding (firm, medium, respectively soft) and FA from the contrecoup regions was compared between the groups. The Kruskall-Wallis test revealed no significant differences between the three foam conditions.

Discussion

In this study, we used an established animal model of mTBI, the impact acceleration rat model of injury, and evaluated the brain white matter microstructure using dMRI seven days post-injury. Imaging findings demonstrated an increase in FA in the injured animals suggesting presence of subtle abnormalities to the white matter as a consequence of the injury.

The impact acceleration model was developed in rats to produce diffuse axonal brain injury without focal brain lesions[16,17]. Morphological studies using light and electron microscopy

have revealed extensive injury to the neurons, axons, and microvasculature[17]. Brain edema was seen in this latter study within 24 hours although it resolved within a few days[17]. This early post-injury period was also explored using dMRI and was reported as changes in FA and Mean Diffusivity (MD), a dMRI marker of edema[19–22]. The dMRI study reported significant decreases in FA during the first 3 days[19] and increases in MD in animals within 60 minutes post-injury, followed by a continuous decrease in MD, which reached a minimum on day 7 and 14[21], suggesting that edema occurs within minutes and lasts for less than 7 days post-injury. In addition, electron microscopy revealed diffuse axonal injury in the form of retraction balls, which was present beyond the time of one week[17], suggesting that reparative and neurodegenerative processes continued to occur in the traumatized white matter. To the best of our knowledge, the accelerated impact rat model has not been explored using the FA measure 7 days post injury and at this period of regeneration and degeneration of brain tissues. In our study, this period of regeneration and degeneration is characterized by increased FA at day 7 post-injury (figure 3).

In the current study, brain regions with increased FA were localized to several long-range white matter tracts. These included parts of the anterior commissure, the cerebellar peduncles, the forceps minor of the corpus callosum, the fimbria of the hippocampus, the fornix, the medial forebrain bundle, the optic chiasm, the pyramidal tract, and spinal trigeminal tract (see figure 2). The original publication of the impact acceleration rat model of injury reported pathogenesis of similar tracts in the first few days of injury, and the presence of retraction balls in the long tracts of the brain stem until day 10 post trauma[17]. Thus the histology and our dMRI findings are consistent with respect to the tracts injured.

The majority of the tracts with increased FA are localized ventrally and represent the contrecoup region of the impact. There were no changes in FA in the region of the impact, in the coup region. As of yet, the mechanism of the coup and contrecoup injury is not well understood[26]. One possible explanation of the injury to the contrecoup region is that it results from an abrupt deceleration of the brain and collision with the skull. There are examples of injury to the contrecoup region such as a result of a falling tree limb hitting the head[27]. While coup and contrecoup injuries are mostly seen in focal injury, we find in this animal model widespread injury to the white matter localized preferentially to the contrecoup region, suggesting that diffuse axonal injury might be restricted to certain areas.

In clinical settings, mTBIs that affect white matter include the brain stem, corpus callosum, cerebral peduncles[28–31], and a number of additional brain areas (e.g., summarized in[11]). The most commonly reported fiber tract with increases in FA is the corpus callosum, reported in several research studies of mTBI patients[32–35] including athletes with repetitive sports-related injuries to the head[36,37]. Our rat model of mTBI seems to reproduce damage to the long-range white matter fiber tracts that are also observed in clinical mTBI in humans.

We also explored impact of height and foam firmness as factor of the severity of injury. We tested for changes in FA in animals exposed to three experimental conditions of height (129 cm, 137.5 cm, 147.5 cm) and of foam padding (firm, medium, soft), but have not found any statistically significant differences. Because of the comparatively small difference between the heights and the foam firmness, and because of the low statistical power of these analyses,

we cannot rule out effects of height or foam in general. Due to the absence of statically significant differences in FA under these experimental conditions in our study, we combined all animals into one group for the analysis of white matter.

This is a pilot study and thus there are several caveats. These caveats include: (i) the small number of animals and the incomplete dose response curve in respect to impact of height and of foam firmness (because the statistical power of these analyses was low, and because of the comparatively small difference between the heights and foam firmness, we cannot rule out effects of height or foam in general)(ii) lack of histology of brain white mater; (iii) the minimal behavioral testing (rats underwent only limited neuroscore testing to confirm mTBI, data not shown); and (iv) the imaging of fixed versus live animals. With respect to the last, there are some reservations regarding the imaging of fixed tissue as opposed to imaging of live animals. This is based on concerns that the process of tissue fixation changes the microstructure and might result in changes in the dMRI parameters. Previously published comparisons of dMRI measures obtained from mouse brains prior (*in vivo*) and post (*ex vivo*) formalin fixation confirms decreases in mean water diffusivity; however water diffusivity anisotropy parameters, such as FA, the measure reported in our study, remained unchanged after fixation[38–40]. The advantage of fixed tissue imaging is the absence of motion artifacts and the unrestricted scanning time.

In summary, this study presents a rodent model of mTBI using a dMRI approach, which is also applied in clinical imaging of humans with mTBI. In the impact acceleration rat model, edema is present in the rat brain within few minutes after injury and resolves within few days, which was reported as changes in the MD[21]. Seven days post injury no changes in MD were detected[21]. However, an electron microscopy study revealed diffuse axonal injury at and beyond the time of the first week pointing to the presence of reparative and neurodegenerative processes [17]. In this study we report increases in FA in the injured animals 7 days post-injury at a time period of tissue reparation. These injuries are localized to the major white matter fiber tracts in the contrecoup region. The finding of increased FA in this animal model is a promising step towards understanding the pathology of mTBI, and developing treatment interventions following mTBI.

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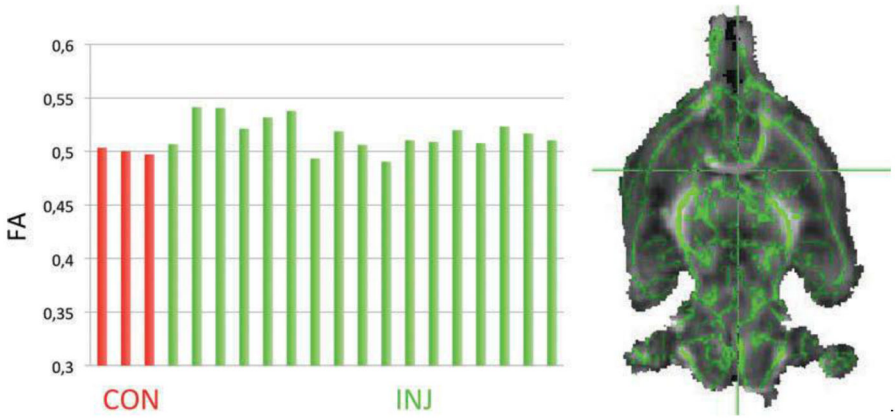

Figure 1. Average FA was extracted from the entire skeleton of each individual animal

The bar graph shows FA values extracted from the sham animals (CON, red bars), and the injured animals (INJ, green bars)(panel left). The image of one animal is presented in horizontal view in the panel on the right. The skeleton of the white matter is colored green. In the background is the FA map of the whole rat brain presented in shades of gray and white. White matter appears in white depicting highest values of FA.

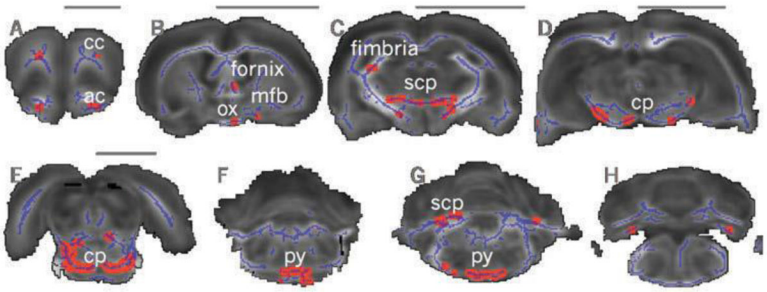

Figure 2. Increases in FA were localized to the contrecoup regions in injured animals

Voxels of increased FA in the injured animals are presented in red. The regions of increased FA were localized to several regions in the ventral part of the brain: in the intrabulbar part of the anterior commissure (aci), the cerebellar peduncles (cp; the inferior peduncle: icp; the superior cp: scp;), the forceps minor of the corpus callosum (fmi); the fimbria of the hippocampus (fimbria); the fornix; the medial forebrain bundle (mfb); the optic chiasm (ox); the pyramidal tract (py) and the spinal trigeminal tract (sp5). The brain white matter was extracted using the TBSS method and is represented as skeleton (blue). The FA map of the whole rat brain is in the background (shades of gray, white matter appears in white depicting high values of FA) on coronal slices (A through H, anterior to posterior views).

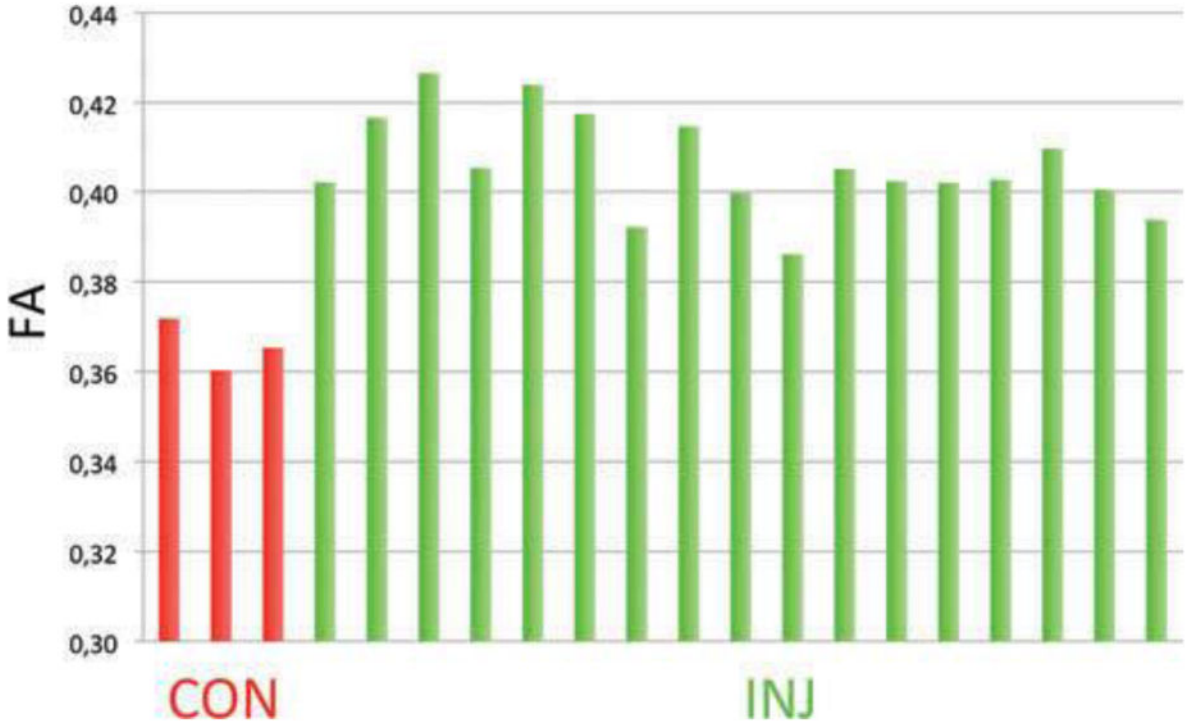

Figure 3. Increased FA in specific areas of the white matter in the injured animals

The average FA in the contrecoup areas was statistically significantly higher in the injured animals (green bars) than in control animals (red bars).