Introduction

Traumatic brain injury (TBI) is the leading cause of acquired disability in young adults, often caused by traffic accidents or sport injuries[1]. Mild TBI (mTBI) is the most common type of TBI. While conventional scans (CT or anatomical MRI) show no evidence of injury due to the diffuse and subtle nature of mTBI, the patients can suffer cognitive defects such as memory problems, attention deficits, and executive control deficits, evens years after their injury. The aim of this study is to investigate whether multishell diffusion MRI analysis can be used to detect microstructural changes in a rat model of mTBI.

Methods

Animal model: Nine female Wistar rats weighing 250 ± 19.6 g sustained mTBI utilizing the Marmarou weight drop model[2]. In brief, in anesthetized rats a steel helmet was fixed on the skull 1/3 before and 2/3 behind bregma. The rat was positioned under a 450 g brass weight on a foam bed. The weight was dropped from a height of 1m guided through a plexiglass column. The foam bed together with the rat was rapidly removed from the column to prevent a second impact.

Imaging and data analysis: MRI data were acquired on a 7T MRI scanner (PharmaScan, Bruker, Ettlingen) before and 1 week after brain injury. T2-weighted images were acquired for anatomical reference. Multishell diffusion data were acquired with multiple directions (b=800, 1500 and 2000 s/mm^2; 32, 46 and 64 directions; 5, 5 and 7 b0 images respectively). DWI images were corrected for EPI, motion and eddy current distortions in ExploreDTI version 4.8.6.[3]. Moreover diffusion kurtosis tensor estimation was performed using weighted linear least squares method[4]. Maps for the diffusion and kurtosis metrics (FA, MD, AD, RD, MK, AK and RK) were calculated based on the diffusion kurtosis imaging model[5] and maps for the white matter metrics (AWF, AxEAD, RadEAD, tortuosity) were calculated based on a white matter diffusion model[6]. The maps were co-registered in SPM12 with an anatomical template based on the local population, using the anatomical T2-weighted images. The template was constructed by realignment, coregistration and normalisation to the first subject. A volume-of-interest analysis was performed in the hippocampus, cingulum and corpus callosum using Amide toolbox[7]. The Wilcoxon signed-rank test was performed for each map to investigate changes in white matter between the two time points in SPSS. $P < 0.05$ was considered significant.

Immunohistochemical analysis: Six rats were sacrificed for histological analysis and perfused with 4% paraformaldehyde. Sections of the brain were stained for the following cellular components: synapses (with anti synaptophysin); myelin (with Lyxol Fast Blue staining); astrocytes (with anti glial fibrillary acidic protein); and neurons (with anti neuronal nuclei, NeuN).

Table 1. Results for the diffusion, kurtosis and white matter metrics before (pre) and after (post) impact in the corpus callosum, cingulum and hippocampus. Abbreviations: AD, axial diffusivity (10^{-3} mm^2/s); AK, axial kurtosis; AWF, axonal water fraction; AxEAD, axial extra-axonal diffusivity (10^{-3} mm^2/s); RadEAD, radial extra-axonal diffusivity (10^{-3} mm^2/s); FA, fractional anisotropy; MD, mean diffusivity (10^{-4} mm^2/s); MK, mean kurtosis; RD, radial diffusivity (10^{-4} mm^2/s).

Metric	Corpus callosum		Cingulum		Hippocampus	
	Pre	Post	Pre	Post	Pre	Post
AD	1,40 ± 0,04	1,47 ± 0,09	1,32 ± 0,04	1,30 ± 0,07	1,15 ± 0,05	1,19 ± 0,46 [a]
AK	0,66 ± 0,06	0,78 ± 0,04 [a]	0,69 ± 0,06	0,75 ± 0,02	0,62 ± 0,07	0,72 ± 0,05 [a]
AWF	0,30 ± 0,02	0,36 ± 0,03 [a]	0,31 ± 0,02	0,35 ± 0,02 [a]	0,24 ± 0, 02	0,27 ± 0,01 [a]
AxEAD	1,84 ± 0,09	2,05 ± 0,16	1,75 ± 0,08	1,81 ± 0, 12	1,43 ± 0,08	1,54 ± 0,69 [a]
RadEAD	1,05 ± 0,05	1,01 ± 0,09	1,02 ± 0,05	9,81 ± 0,61	1,03 ± 0,07	1,07 ± 0,66
FA	0,36 ± 0,04	0,45 ± 0,04 [a]	0,35 ± 0,01	0,39 ± 0,03 [a]	0,20 ± 0,01	0,22 ± 0,03
MD	9,93 ± 0,33	9,67 ± 0,61	9,50 ± 0,31	9,07 ± 0,48	9,34 ± 0,49	9,51 ± 0,35
MK	0,68 ± 0,09	0,84 ± 0,09 [b]	0,73 ± 0,09	0,88 ± 0,07	0,54 ± 0 08	0,67 ± 0,07 [b]
RD	7,91 ± 0,33	7,15 ± 0,62	7,66 ± 0,30	7,09 ± 0,41 [b]	8,29 ± 0,49	8,32 ± 0,39

a. p = 0,028; b. p = 0,046

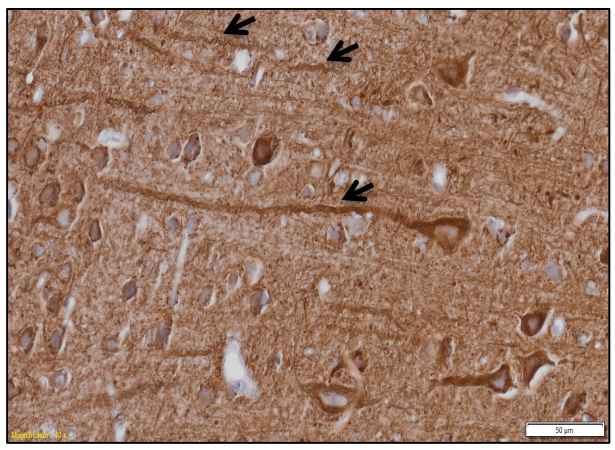

Figure 1. Immunostaining with anti neuronal nuclei (NeuN). Arrowheads indicate injured axons in the cortex.

Results

Due to outliers for the radial kurtosis and tortuosity metric, these metrics were removed from final analyses. As can be seen from Table 1, we found increased values of several DTI, DKI and WMTI metrics (AD, AK, AWF, AxEAD, FA, MD, MK) in the three regions of interest in the post-scan compared to the pre-scan. Immunohistological staining for NeuN might suggest that several neurons are undergoing Walerian degeneration.

Discussion and conclusion

An increase in AWF could be explained by axonal swelling. Furthermore an increase in AK, MK and FA supports this hypothesis. Since the AWF was increased in all three regions we can conclude that this metric is very sensitive for changes in microstructure due to mTBI, 7 days post injury. Walerian degeneration in the cortex might indicate injured and swollen axons and can be proof of concept that our model induces traumatic brain injury. Further histological analysis is currently on going in order to provide a biological basis to support this hypothesis.

References

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