Characterizing microstructural alterations in a mTBI ratmodel: a multi-shell diffusion MRI analysis

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Introduction

Traumatic brain injury (TBI) is the leading cause of acquired disability in young adults, often caused by traffic accidents or sport injuries (Thurman, 2016). 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 from chronic cognitive defects evens years after their injury. Due to the lack of specificity of FA for histological features (Jones et al, 2013), the aim of this study is to better characterize white matter changes with advanced diffusion MRI analysis in a rat model of mTBI.

Methods

Animal model: 20 female Wistar rats weighing 265±16.9g were used in this study. 13 sustained mTBI utilizing the Marmarou weight drop model (Marmarou et al, 1994) and 7 received a sham injury. 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 450g 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. For the sham animals the procedure was the same accept for the impact.

Imaging and data analysis: MRI data were acquired on a 7T MRI scanner (PharmaScan, Bruker, Ettlingen) before, 1 day, 1 week and 3 months after injury. T2-weighted images were acquired for anatomical reference. Multi-shell diffusion data were acquired with multiple directions/b-values, i.e. b=800, 1500 and 2000 s/mm^2; 32, 46 and 64 directions. DWI images were corrected for EPI, motion and eddy current distortions in ExploreDTI version 4.8.6. (Leemans et al, 2009). Moreover diffusion kurtosis tensor estimation was performed using weighted linear least squares method (Veraart et al, 2013). Maps for the diffusion and kurtosis metrics (FA, MD, AD, RD, MK, AK and RK) were calculated based on the diffusion kurtosis imaging model (Veraart et al, 2011) and maps for the white matter metrics (AWF, AxEAD, RadEAD, tortuosity) were calculated based on a white matter diffusion model (Fieremans et al, 2011). The maps were then co-registered in SPM12 on an anatomical template based on the local population, using the T2-weighted images. Next, a volume-of-interest analysis was performed in the hippocampus, cingulum, cortex and corpus callosum using Amide toolbox (Loening et al, 2003). Bad quality scans were left out of the analysis. The Wilcoxon signed rank test was performed on each map to investigate changes in white matter between time points in SPSS. Subsequently, the Mann-Whitney U test was carried out to test for differences between the sham and TBI group at each timepoint. $P<0.05$ was considered significant.

Histological analysis: Rats were sacrificed for histological analysis and perfused with 4% paraformaldehyde 1 day (n=1), 1 week (n=6) and 3 months (n=10) after impact. Sections of the brain were stained for the following markers: synapses (with anti-synaptophysin); myelin (with Luxol Fast Blue staining); astrocytes (with anti-glial fibrillary acidic protein); and neurons (with anti-neurofilament, NF).

Results

As can be seen from Table 1, we found increased values for AK and AWF, 1 week post injury compared to baseline in the TBI group for the corpus callosum. The increase in AWF was also seen in the cingulum. When comparing the TBI and sham group, a significant difference was found between the two groups for the AWF, 1 week post injury in the cingulum. No significant differences were found 1 day and 6 months post injury or in the sham group. Staining for NF might suggest that several neurons are undergoing Walerian degeneration (1 week after impact)(Figure 1).

Table 1. Results for the TBI group for the axial kurtosis (AK) and axonal water fraction (AWF) of the corpus callosum and cingulum at (baseline)(n=13) and 1 week (n=11) after impact and respective p-values according to the Wilcoxon signed rank test.

Metric	Corpus Callosum			Cingulum		
	baseline	1 week	p-value	baseline	1 week	p-value
AK	0.75 ± 0.01	0.81 ± 0.01	0.003	0.74 ± 0.02	0.80 ± 0.005	0.091
AWF	0.35 ± 0.01	0.37 ± 0.005	0.05	0.33 ± 0.007	0.35 ± 0.003	0.047

Discussion and conclusion

An increase in AWF could be explained by axonal swelling, consistent with an increased AK. Because AWF values were significantly different between the TBI and sham group as well, we can conclude that this metric is very sensitive for changes in microstructure due to mTBI (1 week post injury). Since there were too few subjects 1 day and 6 months post injury in each group (n=3) and the variability of the measurements was too high, we could not find significant results at these time points. More subjects will be added in future research. Walerian degeneration in the cortex might indicate injured and swollen axons. Further histological analysis is needed in order to provide a biological basis to support this hypothesis.

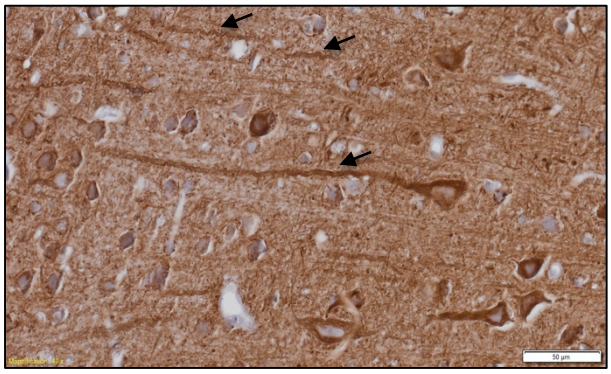

Figure 1. Immunostaining with anti neurofilament (NF) 1 week after impact. Arrowheads indicate injured axons in the cortex.

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