# Characterization of Cortex and White Matter Injury in a Mild Hypoxic-Ischemic Neonatal Rat Model by Diffusion Tensor MR Imaging

**S. Wang[1], E. X. Wu[2,3], H-F. Lau[2,3], and P-L. Khong[1]**

[1]Department of Diagnostic Radiology, The University of Hong Kong, Hong Kong, Hong Kong, Hong Kong, [2]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong, [3]Department of Electrical and Electronic Engineering, The University of Hong Kong

**Introduction:** Mild hypoxic-ischemic (HI) neonatal brain injury is known to cause astrogliosis and radial glial disruption to the cortex[1] and dysmyelination to the WM[2]. We apply diffusion tensor MR imaging (DTI) to evaluate the changes in the cortex and WM of a mild HI neonatal rat brain injury model. We hypothesize that the quantitative indices of DTI are able to reflect the histological changes of HI injury, namely, astrogliosis in the cortex and dysmyelination in the WM.

**Materials and Methods:** Seven-day-old rats underwent unilateral left common carotid artery ligation followed by exposure to 8% oxygen-balanced nitrogen for 50 minutes ($n=9$) in order to create mild HI induced brain damage[2]. DTI was performed using a 7T NMR scanner (Bruker, Germany) and microimaging mouse brain coil on 24h post HI. Images were obtained in the coronal plane using the following parameters: FOV=32mm$^2$, TR/TE=3000ms/32ms, matrix size 128 x 128, slice thickness=0.5mm. FA, trace, $\lambda_{//}$ and $\lambda_{\perp}$ maps were created for quantitative analysis by using DTIstudio (Johns Hopkins University, U.S). ROIs were manually drawn over the primary somatosensory cortex of each hemisphere on the FA, trace, $\lambda_{//}$ and $\lambda_{\perp}$ maps on two consecutive slices (Bregma -1.08mm and Bregma -2.4mm)[3] (Fig 1). ROIs were also manually drawn over the external capsule (EC) of each hemisphere on five consecutive slices[2] (Fig 1). Paired t test was used to detect statistical differences in the DTI indices between the injury/control cortex and EC. Rats were randomly sacrificed ($n=3$) for histological analysis of the morphological characteristics of cortex and EC (H&E stain), myelin in the EC by using Luxol fast blue (LFB) stain and astrocytes in the cortex by using immunohistochemistry analysis of glial fibrillary acidic protein (GFAP) (SMI-22, 1:1000, USA).

**Results: Comparison of DTI indices between injury and control cortex (Table 1):** FA was significantly decreased in the injured cortex compared to the control cortex with a reduction of 11.7%, ($p<0.01$). A significantly decreased $\lambda_{//}$ was found in the injured cortex compared to control cortex with a reduction of 5.8%, ($p<0.05$). However, there were no significant differences in $\lambda_{\perp}$ and trace. **Comparison of DTI indices between injury and control EC (Table 1):** FA was significantly decreased in the injured EC compared to control EC with a reduction of 10.1%, ($p<0.01$). Significantly increased $\lambda_{\perp}$ (13.5%, $p<0.01$) and trace (11.0%, $p<0.01$) were demonstrated in the injured EC compared to control EC. However, $\lambda_{//}$ was similar in both sides of EC. **Histological evaluations of cortex (Fig 2):** H&E stain showed scattered pyknotic cells and a slight edema in the injured cortex but without necrosis. GFAP stain showed the numbers of GFAP-immunoreactive cells were markedly increased with larger cell bodies in the injured cortex compared to control cortex in keeping with astrogliosis. **Histological evaluations of EC (Fig 3):** H&E stain showed mild vacuolation changes in injured EC but without necrosis. Much weaker LFB staining intensity was demonstrated in the injured EC compared to control EC in keeping with reduced myelination.

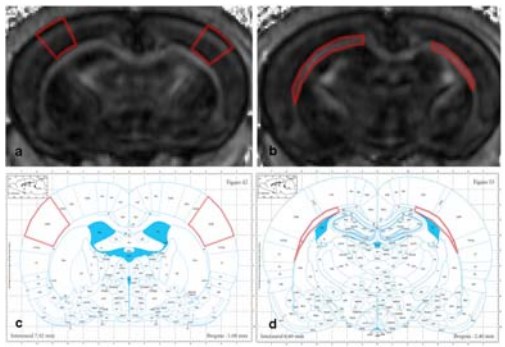

Fig 1. (a) shows ROI manually drawn over the primary somatosensory cortex (red area) on the FA map which is corresponding to rat brain at Bregma -1.08mm (c). (b) shows ROI drawn over the EC on the FA map (red area) which is corresponding to rat brain at Bregma -2.4mm (d)[3].

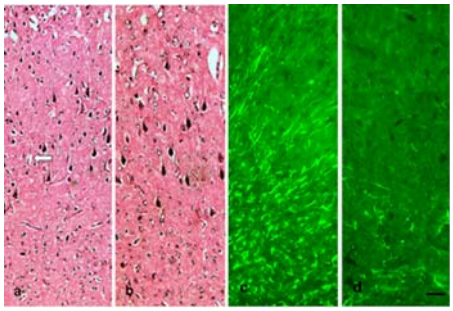

Fig 2. Histology findings in the cortex: H&E stain (a-b). Injured cortex (a) shows scattered pyknotic cells (arrow) compared to normal appearance in control cortex (b). GFAP stain: (c-d) shows much increased GFAP positive cells in the injured cortex (c) compared to control cortex (d). (Scale bar =25μm).

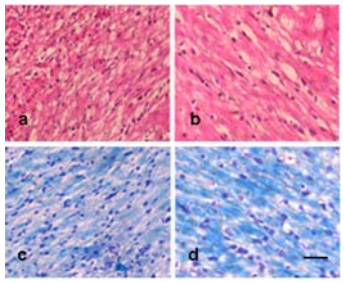

Fig 3. Histology findings in the EC: H&E stain (a-b): Injured EC (a) shows vacuolation changes compared to normal appearance in control EC (b). LFB stain (c-d): injured EC (c) shows decreased LFB stain intensity compared to control EC (d). (Scale bar =25μm)

**Conclusion:** Our findings suggest that DTI indices can characterize mild HI induced injury in the cortex and WM; significantly decreased FA and $\lambda_{//}$ with no change in $\lambda_{\perp}$ appears to characterize astrogliosis in the cortex whilst significant reduction of FA with increased $\lambda_{\perp}$ and trace characterize dysmyelination in the WM. It has been proposed that reactive astrogliosis imposes diffusion barriers due to hypertrophy of astrocytic processes, although how this affects $\lambda_{//}$ is not well understood[4]. Dysmyelination causes increased water diffusion perpendicular to the axon, therefore increased $\lambda_{\perp}$ but decreased FA. Combined analysis of DTI indices may be useful potential markers for the non-invasive monitoring of mild HI induced brain injury.

Quantitative analysis of DTI indices in mild HI neonatal rat model at 24h post HI ($n=9$)

| | Primary somatosensory cortex | | | | External capsule | | | |
|---|---|---|---|---|---|---|---|---|
| | Injury | Control | Δ% | p | Injury | Control | Δ% | p |
| **FA** | **0.21±0.03** | **0.24±0.02** | **-11.7** | **<0.01** | **0.29±0.04** | **0.32±0.04** | **-10.1** | **<0.01** |
| **Trace** | 2.11±0.27 | 2.17±0.11 | -2.9 | 0.19 | **2.72±0.19** | **2.45±0.15** | **11.0** | **<0.01** |
| **$\lambda_{//}$** | **0.86±0.12** | **0.91±0.04** | **-5.8** | **0.02** | 1.14±0.10 | 1.13±0.09 | 0.98 | 0.13 |
| **$\lambda_{\perp}$** | 0.62±0.08 | 0.63±0.04 | -0.3 | 0.82 | **0.77±0.05** | **0.67±0.04** | **13.5** | **<0.01** |

Table 1: DTI quantitative indices of injury and control primary somatosensory cortex and EC at 24h after mild HI in a neonatal rat model. Values are shown in mean±sd. Trace, $\lambda_{//}$ and $\lambda_{\perp}$ are in units of μm$^2$/ms. Δ%=percentage of variation in DTI indices of injury side compared with control side. $p<0.05$ are in bold

**References:** 1. Sizonenko SV et al. Cereb Cortex 2007;17:2609-17. 2.Wang S et al. Stroke 2008;63:5950-6. 3. Paxinos, G., and Watson, C. The Rat Brain in Stereotaxic Coordinates. 2007;p85-96. 4. Vorisek I et al. MRM 2002; 48:994-1003.