Characterization of early hypoxic-ischemic injuries to the neonatal mouse cerebral cortex and hippocampus using diffusion tensor imaging

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Introduction: Hypoxic-ischemic (HI) injury to the premature neonatal brain often induces damages to the white and gray matter structures causing significant morbidity and mortality. The Rice-Vannucci mouse model of HI is commonly used for studying the pathology and treatment of this injury. In this model, the cerebral cortex and hippocampus are primary targets of HI related injury, and the injured tissues often degrade into porencephalic cysts 2-3 weeks after the initial injury. Diffusion MRI and diffusion tensor imaging (DTI) have been used to study HI related gray matter and white matter injuries in patients (1, 2) and animal models (3, 4). DTI can visualize the radial organization of radial glial cells and axons in the neonatal rodent cerebral cortex and pyramidal cell layer in the hippocampus (4, 5), and can detect early cortical injury and disruption of the radial pattern in a rat model of HI (4). In this study, we used *ex vivo* DTI and immunohistochemistry to study early cortical and hippocampal injuries in the mouse model of neonatal hypoxic-ischemia.

Methods: The Rice Vannucci model (unilateral carotid ligation + 45 minutes of hypoxia FIO_2=0.08) was used to cause brain injury in postnatal (p)7 C57BL6 mice. Control and injured mice were sacrificed at p8, p11, p15 and p42 ($n \geq 5$ in each group at each stage). *Ex vivo* DTI was performed on a 9.4 T scanner with a resolution of 0.1 mm x 0.1 mm x 0.1 mm (b = 1600 s/mm^2, Δ = 15 ms, δ = 6 ms). For p8 – p15 mouse brains, the DTI data were co-registered to a standard template using nonlinear normalization, and average tensor data for each stage were generated. We calculated the angle (α) between the primary eigenvector (V_1) of each normalized brain and the V_1 of the average brain at each pixel as a measure of change in tissue diffusion orientation. Images from p42 mouse brains were normalized by affine transformation, and the spatial distributions of porencephalic cyst as defined in the diffusion weighted images were calculated. Regions of interest (ROIs) were defined in the sensory cortex, motor cortex and pyramidal cell layers of the hippocampus, and changes in fractional anisotropy (FA) and orientation (angle α) in these ROIs between the control and injured mice were examined (nonparametric Wilcoxon rank sum tests with a threshold for significance of 0.05 after corrections for multiple comparisons).

Results and Discussions: In the injured p42 mice, the regions of the ipsilateral sensory cortex (SCX) and pyramidal cell layer (Py) in the hippocampus have high probability of forming porencephalic cyst following HI at p7. In uninjured p8 mice, SCX and Py have relatively high FA (0.24 ± 0.03 and 0.30 ± 0.03, respectively) and unique radial pattern (Fig. A control), which may be due to the existence of radial glial or axonal and dendritic networks. Twenty-four hours after HI injury, injured SCX and Py have reduced FA and disruption of the radial pattern, which is visualized in the angle (α) maps (Fig. A Injured). ROI based analysis (dashed lines in Fig. A) revealed significant differences in FA and tissue orientation (angle) between control and injured animals (Fig. B-C). Injury related changes in FA and tissue orientation could also be observed in SCX and Py at p11 and p15. Robust degeneration of neurons and axons/dendrites in the injured cerebral cortex and hippocampus at p8 were revealed by Fluoro Jade (Fig. D), silver, neurofilament-M (Fig. E) and MAP2 staining. Neurofilament-M and MAP2 show marked accumulation of these proteins, which normally reside in the axonal and dendritic compartments, within neuronal soma, suggesting abnormal trafficking, and defining injury to the axonal and dendritic compartments (Fig. E). The results suggest that changes in DTI signals may reflect the degree of underlying neurodegeneration and disruption of the axono-dendritic compartment in the cerebral cortex and hippocampus.

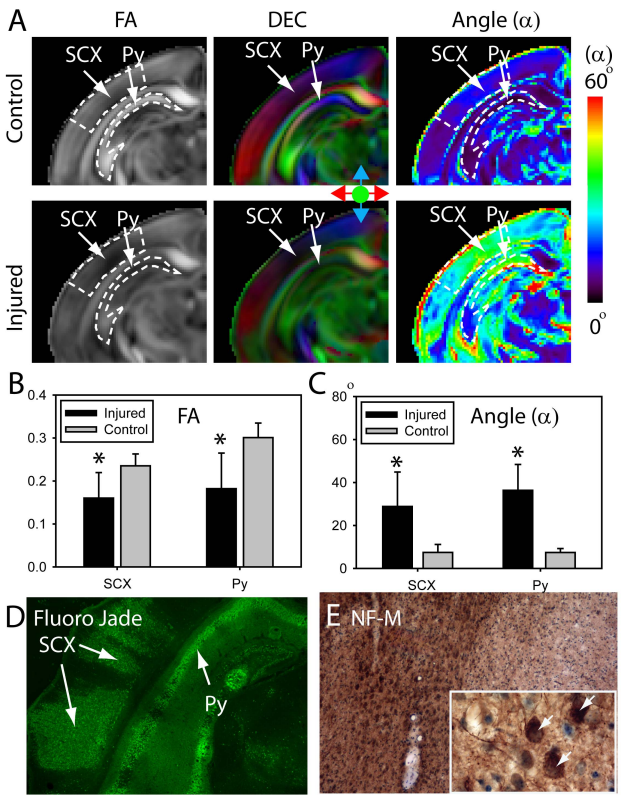

Fig. 1: A: Average maps of FA, direction-encoded-colormap (DEC), and changes in orientation (angle) of control and injured p8 mouse brains (n=6). The dashed lines illustrated the ROIs for the sensory cortex (SCX) and pyramidal layer (Py). **B-C:** Changes in FA and angles in SCX and Py at p8. * = $p < 0.01$. **D:** Fluoro Jade staining shows degenerating neurons in injured P8 mouse cortex and hippocampus. **E:** Neurofilament (NF-M) staining shows injured cerebral cortex. The high magnification insert shows accumulation of neurofilaments in the cell bodies (indicated by white arrows).

References: **1.** Huppi, P.S., et al. Pediatrics, 2001. 107(3): p.455-60. **2.** Ward, P.S., et al. Pediatrics, 2006. 117(4): p.e619-30. **3.** Aden, U.V., et al. Stroke, 2002, 33(5): p.1405-10. **4.** Sizonenko, S.V. et al. Cerebral Cortex, 2007. 17(11): p. 2609-17. **5.** Zhang, J. et al. Neuroimage, 2001. 15(4): p.892-901.