Underlying mechanisms of Apparent Diffusion Coefficient changes in the stroke rat brain revealed via diffusion basis spectrum imaging and histological examinations

Yi-Hua Hsu[1], Chien-Hsiang Huang[1], Chiao-Chi V. Chen[1], Yong Wang[2], Peng Sun[2], Sheng-Kwei Song[2], and Chen Chang[1]

[1]*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, Taiwan,* [2]*Department of Radiology, Washington University School of Medicine, St. Louis, MO, United States*

Target audience: Researchers in stroke and diffusion MRI.

Purpose: Diffusion tensor imaging (DTI) has been widely used for evaluating tissue injuries caused by ischemic stroke [1], detecting and characterizing edema formation, axonal injury, and demyelination [2]. However, DTI derived apparent diffusion coefficient (ADC) loses its sensitivity and specificity to tissue lesions with increasing pathological and anatomical complexity. Herein, *in vivo* diffusion basis spectrum imaging (DBSI) [3] and post-imaging histology were employed to characterize the pathological and structural changes underlying DTI features in a rat model of transient middle cerebral artery occlusion (MCAO).

Methods: Male Sprague-Dawley rats (300-350g, n=4) underwent transient MCAO for 60 minutes. Rats were scanned longitudinally for one, three, seven, and 35 days after MCAO followed by post-MRI histology. All MRI experiments were performed on a 7T scanner (PharmaScan 70/16, Bruker, Germany). The DBSI analysis was performed by fitting the 99 diffusion weighted signals using a linear combination of diffusion basis set consisting of cylindrically symmetric diffusion tensors [3] with the freedom to vary $\lambda_{\parallel}$ and $\lambda_{\perp}$ to estimate the number of anisotropic diffusion tensor components and the associated principal directions. After the number of anisotropic diffusion tensors was computed, the number of isotropic component was further determined using nonnegative least-squares (NNLS) analysis [3]. The global nonlinear optimization was conducted employing direct pattern search to solve DBSI. A traditional derivative based optimization method was employed following the global optimization to improve the accuracy of the solution. Indices from DBSI include axial and radial diffusivity of the detected fiber, the ratio of cell and water components etc. [3].

Results and Discussion: The temporal evolution of ischemic injury in the rat brain after MCAO was evaluated by T2WI, DTI, and DBSI. In gray matter (Fig 1), conventional T2 maps show gradual increase of T2 values in the ipsilateral cortex from Day 1 to Day 35, reflecting the ischemia induced increase of water content. The DTI derived ADC of ischemic cortex initially decreased on Days 1 & 3, and increased on Days 7 & 35. Results suggested that the water diffusion in the ipsilateral cortex was initially restricted but became progressively less hindered. Notably, regionally decreased T2 and ADC (arrowhead) within the infarcted ipsilateral cortex was seen on Day 7 that was absent on Day 35. To understand the evolution of brain water diffusion after MCAO, DBSI analysis was also performed to assess the distribution of cortical ADC reflecting free, hindered, and restricted diffusion, respectively. The ADC fraction maps from DBSI show high percentage of restricted diffusion water (arrow) in the ipsilateral cortex on Days 1 & 3. On Days 7 & 35, the percentage of hindered diffusion water increased in the ipsilateral cortex. The percentage of water content which shifted from being restricted to hindered, and free, as shown by DBSI, parallels the evolution of DTI derived cortical ADC. On Day 7, high percentage of hindered water (arrowhead) was observed within the ipsilateral cortex, coinciding with the region of decreased T2 and ADC values. In the external capsule (Fig 2), fractional anisotropy (FA), axial diffusivity, and radial diffusivity maps derived using DTI consistently showed subtle changes (arrow). DBSI derived residual fiber axial and radial diffusivity decreased (arrowhead) on Day 35. Increased immunostaining of MAP2 (marker of dendrites) was seen in the ipsilateral cortex on Day 1 in comparison with the contralateral cortex (Fig 3). From Day 3, MAP2 immunoreactivity gradually fractured and eventually disappeared on Day 35 due to increased tissue destruction. The pattern of MAP2 immunoreactivity was consistent with the early decreased ADC and chronically increased ADC and the evolution of damages of neuronal processes. H&E showed significant cell infiltration and/or proliferation in the ipsilateral cortex on Day 7 (Fig 4), consistent with the high percentage of hindered water component seen by DBSI. Quantitative analysis of the external capsule is currently being pursued to compare DBSI estimated axonal injury and demyelination on Days 1, 3, 7, and 35 with immunohistochemistry.

Conclusion: We demonstrated that *in vivo* DBSI is capable to directly detect the ischemia-induced pathologies, detecting, differentiating, and quantifying tissue injury and cell infiltration/proliferation.

References: [1] Sotak CH et al. *NMR Biomed.* 2002. [2]Song S.K. et al. *Neuroimage.* 2003. [3] Wang,Y. et al. *Brain*. 2011.

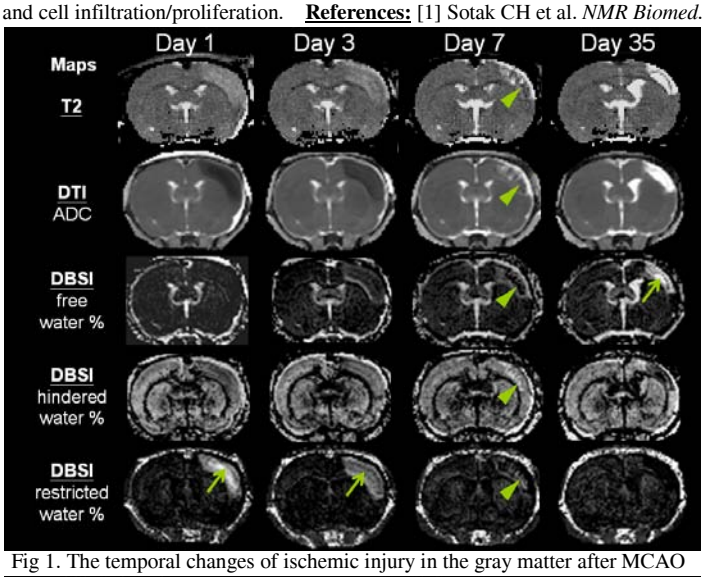

Fig 1. The temporal changes of ischemic injury in the gray matter after MCAO

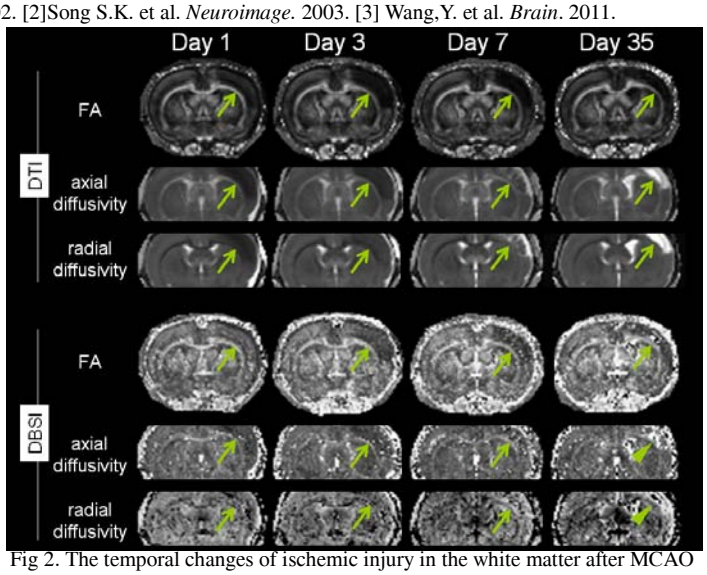

Fig 2. The temporal changes of ischemic injury in the white matter after MCAO

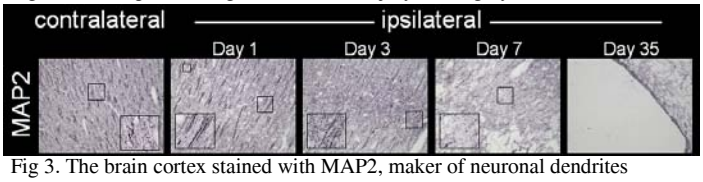

Fig 3. The brain cortex stained with MAP2, maker of neuronal dendrites

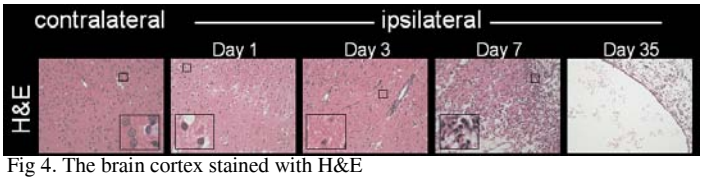

Fig 4. The brain cortex stained with H&E

Acknowledgements: Supported in part by NIH R01-NS047592, P01-NS059560, NMSS RG 4549A4/1, and DOD W81XWH-12-1-0457