

# Evaluation of Vascular and Functional Recovery in Focal Ischemic Rats Using Functional MRI

Shiliang Huang<sup>1</sup>, Qiang Shen<sup>1</sup>, Fang Du<sup>1</sup>, Yen-Yu I Shih<sup>1</sup>, and Timothy Q Duong<sup>1</sup>

<sup>1</sup>Research Imaging Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

**INTRODUCTION** Recovery of perfusion and/or diffusion abnormalities does not necessarily indicate functional and metabolic recovery in stroke. The ability to evaluate vascular coupling and functional status following ischemic brain injury during the acute and chronic phase would add another useful tool to the stroke imaging protocol. Carbon dioxide is a potent vasodilator and plays an important role in modulating cerebral blood flow (CBF). It can be used to assess the integrity of the neural-vascular coupling in the brain. Forepaw stimulation is widely accepted model for fMRI studies in rats.

In this study, we developed a protocol for combined diffusion, perfusion and functional MRI to investigate transient focal ischemia in rats during the acute and chronic phase. Quantitative perfusion and diffusion imaging was performed and an improved iterative self-organizing data (ISODATA) cluster analysis was used to classify normal, ischemic core and “diffusion-perfusion mismatch” tissues. BOLD and CBF fMRI was used to longitudinally evaluate the hypercapnic and forepaw-stimulation responses in different brain regions and in the forepaw somatosensory cortices up to one month post-ischemia.

**METHODS** Four male Sprague-Dawley rats (300-350g) were anesthetized with 2% isoflurane during stroke surgery. Transient (45 min) focal brain ischemia was induced in the right hemisphere using the MCAO method [1]. Isoflurane was switched to ~1.2% during imaging. Rats were mechanical ventilated. Body temperature, respiration rate, heart rate and blood oxygen saturation level were continuously monitored and maintained within normal ranges.

MRI was performed on a Bruker 7T/40cm magnet. Diffusion and perfusion images were acquired with single-shot EPI, matrix = 96x96, FOV = 2.56x2.56 cm<sup>2</sup>, seven 1.5mm slices every 30mins for 4 hours. Two trials of hypercapnic challenge and 6 trials of forepaw stimulation were performed after reperfusion. Hypercapnic challenge used 5% CO<sub>2</sub> with air, acquired using continuous arterial spin labeling (cASL) sequence with TR = 3s and TE = 10ms. Two forepaws were stimulated simultaneously in series using 3 mA, 1.0 ms pulse duration and 12 Hz. Forepaw stimulation fMRI were acquired using gradient-echo EPI with TR = 1s and TE = 25ms. Stimulation paradigm was 45s rest, 15s stimulation and 4 epochs. Rats were also scanned on day-1, day-2, day-7 and day-28 post-occlusion.

Apparent diffusion coefficient (ADC) and CBF maps were calculated [2]. An improved ISODATA clustering algorithm [3] was used to cluster the tissues to normal, perfusion-diffusion mismatch and ischemic core in right hemisphere based on basal CBF and ADC data before reperfusion. CBF fMRI activation maps and absolute CBF changes were calculated for each tissue type associated with CO<sub>2</sub> challenge. BOLD fMRI activation maps and percent changes were calculated for the forepaw somatosensory cortices associated with forepaw stimulation.

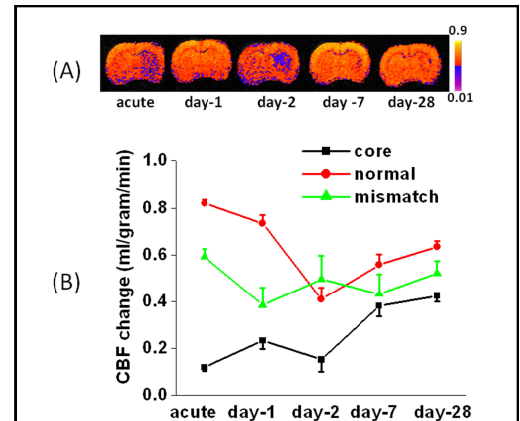
**RESULTS** Cross correlation maps of CBF responses to CO<sub>2</sub> challenge at different time points of a representative rat was shown in **Figure 1(A)**. Group-averaged absolute CBF changes induced by CO<sub>2</sub> challenge for normal, mismatch and core tissue were plotted in **Figure 1(B)**. CO<sub>2</sub> induced significant CBF increases in the normal hemisphere as expected. Mismatch tissue showed stable and normal CO<sub>2</sub> responses throughout. The CO<sub>2</sub> challenge response of core tissue were low or negative in acute phase, recovered a slightly on day-1, but worsened on day-2, and recovered on day-7 and -28. Notice that normal tissue also had the worst response to CO<sub>2</sub> challenge on day-2.

**Figure 2** showed the cross correlation maps of forepaw stimulation BOLD responses. Forepaw stimulation response of lesion side was markedly attenuated at acute phase, increased monotonically from day-1 to day-7 and decreased again on day-28 (**Figure 3**). Group-averaged day-2 T2-W images (**Figure 4**) showed striatal lesion.

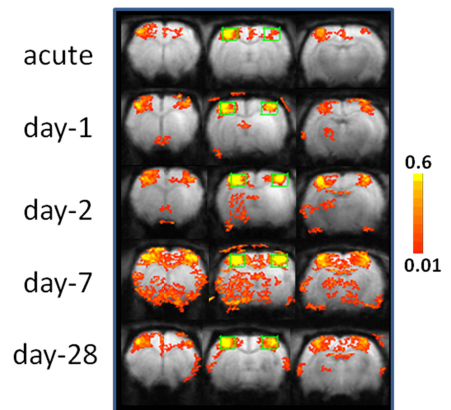
**DISCUSSION** Vascular and functional recovery following transient focal ischemic injury was evaluated, respectively, using hypercapnic challenge and forepaw electrical stimulation fMRI in acute and chronic phase (up to 28 days post-occlusion). Mismatch tissue showed normal CO<sub>2</sub> response throughout post-reperfusion period, indicating fast and completely vascular recovery. Core tissue showed slow, but eventually recovered vascular response. Worst CO<sub>2</sub> responses on day-2 are consistent with the peak of edema and post-ischemic hyperperfusion. Forepaw stimulation responses showed transient recovery and which is most likely because of slow function failure of thalamic tissue (**Figure 4**), which is the major relay of somatosensory cortex.

**CONCLUSION** This study presented a robust function MRI protocol to evaluate vascular and functional recovery following transient ischemic injury. It has the potential to help in the performance evaluation of therapy in various neurological diseases.

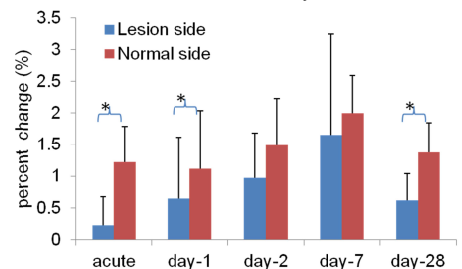
**References** [1] Li F et al, Ann Neurol, 1999; 46:333–342. [2] Shen et al., JCBFM 2003, 23:1479. [3] Shen et al., JCBFM 2004, 24: 887.



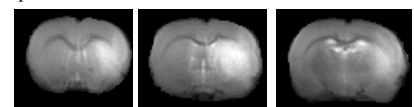
**Figure 1** (A) Cross correlation maps of CBF responses to CO<sub>2</sub> challenge of a representative rat. (B) Group-averaged CBF changes of different tissue



**Figure 2** Cross correlation maps of forepaw stimulation and ROIs used for analysis



**Figure 3** Group-averaged BOLD percent changes of forepaw stimulation



**Figure 4** Group-averaged T2-W image of day-2