Diffusion, Perfusion and Functional Imaging of Transient Ischemic Injury During the Acute Phase

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Introduction: The ability to *chronically* perform fMRI in stroke animal models could have many important applications. Essentially all forepaw stimulation fMRI experiments in rats use α-chloralose anesthetic, which requires terminal experiments. We recently developed a forepaw stimulation model under isoflurane anesthesia on spontaneously breathing rats for chronic fMRI studies [1]. Herein, we extended this approach to evaluate hypercapnic challenge and functional response to forepaw stimulation in *transient* (15 min) focal brain ischemia during the acute phase and again at 24hrs. ADC and CBF images were acquired to characterize ischemic tissue types. BOLD and CBF fMRI were acquired to evaluate CO_2 challenge and forepaw stimulation. $CMRO_2$ changes were calculated using the biophysical BOLD model.

Methods: Six male SD rats (300-350g) were anesthetized with 2% isoflurane during stroke surgery, the left femoral artery was catheterized and needle electrodes were inserted under the skin of the forepaws. Transient (15 min) focal brain ischemia was induced in the right hemisphere (RH) using the MCAO method [2]. Reperfusion was performed on bench top. The rats were immediately put it the magnet for imaging after reperfusion. Isoflurane was switched to ~1.2% during imaging. Rats breathed spontaneously without mechanical ventilation. Hypercapnic challenge used 10% CO_2. Two forepaws were stimulated simultaneously in series using 6 mA, 0.3 ms pulse duration at 3 Hz, previously optimized for isoflurane anesthetic without inducing significant changes in MABP [1].

Diffusion, perfusion and functional images were acquired on 4.7T/40cm magnet with single-shot EPI, matrix = 64x64, FOV = 2.56x2.56 cm^2, eight 1.5 mm slices at 30, 90, 180 mins and 24 hrs. ADC_{ave} was measured with TE = 37 ms, TR = 2 s, b = 5, 1200 s/mm^2 along three principle axes separately, and 16 averages. Combined CBF and BOLD measurements were made using the continuous arterial spin-labeling technique, with parameters similar to the ADC measurement except TE = 20 ms and 90 pairs of images. One trial of hypercapnic challenge and three repeated trials of forepaw stimulation were presented (4 mins baseline and 2 mins of stimulation) approximately every 30 mins. A 4-min break was given between trails.

ADC_{ave} images were calculated. CBF images were calculated using the baseline data of the fMRI measurements. BOLD and CBF fMRI maps and percent changes were calculated for the forepaw somatosensory cortices associated with forepaw stimulation and CO_2 challenge. $CMRO_2$ were also calculated using Davis's biophysical BOLD model as described elsewhere [3,1].

Results and Discussion: Diffusion and perfusion images from one animal are shown in **Fig. 1a**. In general, all animals showed hypoperfusion and most animals showed ADC reduction in the ischemic RH at the first achievable imaging time points (30 mins, MRI data acquisition lasting from 30 to 60 mins). Some animals showed heterogeneous, persistent but midler hypoperfusion at 180 mins despite reperfusion. However, remarkably, 15-min occlusion under isoflurane did not cause persistent ADC lesions at 180 mins in essentially all animals and only a few animals developed very small T_2 and TTC lesions at 24 hrs. Thus, it was concluded that most of the tissues were salvaged following reperfusion. The small infarct size with the 15-min MCAO might have been due to the the neuroprotective effect of isoflurane, which was used during stroke induction in contrast to our earlier studies using chloral hydrate. However, this choice of anesthetic did not affect the overall goal and conclusion of this study.

Hypercapnic challenge at 180 mins and 24 hrs showed no significant attenuation of fMRI responses in the ischemic RH relative to the normal LH **(Fig 1b)**. Similarly, forepaw stimulation at 180 mins and 24 hrs also showed no significant attenuation of fMRI responses in the ischemic RH relative to the normal LH. The group-average data of CO_2 and forepaw-stimulation CBF and BOLD responses in the primary somatosensory cortices are summarized in **Fig. 2**. There were no statistical differences in *basal* CBF and *basal* T_2-weighted signal intensities between LH and RH somatosensory cortices ($P > 0.05$). Similarly, there were also no statistical differences in *stimulus-evoked* CBF and BOLD fMRI responses between LH and RH somatosensory cortices ($P > 0.05$).

Table 1 summarized the M values and stimulus-evoked $CMRO_2$ changes in the primary somatosensory cortices in the LH and RH at different time points post occlusion. M values in the LH and RH were not statistically different and they ranged from 7.7% to 9.4% across different time points, consistent with that (*ca.*, 5%) reported previously with a slightly shorter TE of 15 ms in normal rats [1]. Forepaw-stimulation induced $CMRO_2$ increases in the LH and RH were also not statistically different and they ranged from 13 to 21% across different time points, consistent with, but slightly lower than, that of 24% increase reported previously [1].

Conclusion: This study demonstrated a non-invasive imaging protocol in which perfusion, diffusion and functional (BOLD, CBF, and $CMRO_2$) imaging can be performed in a single setting with a 30-min temporal resolution. The choice of isoflurane as an anesthetic makes it possible to perform these imaging studies in chronic fashion without sacrificing animals, in contrast to the use of choral hydrate or a-chloralose anesthetics.

References [1] Liu et al., MRM 2004, 52:277. [2] Shen et al., JCBFM 2003, 23:1479. [3] Davis et al., PNAS 1998, 95:1834.

Fig. 1a

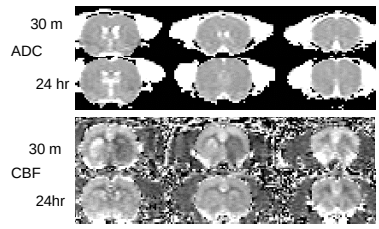

Fig. 1b

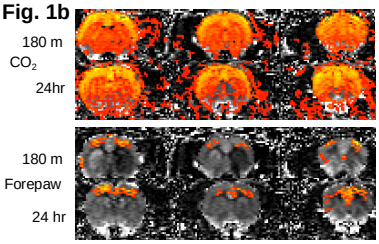

Fig. 2a. CO_2

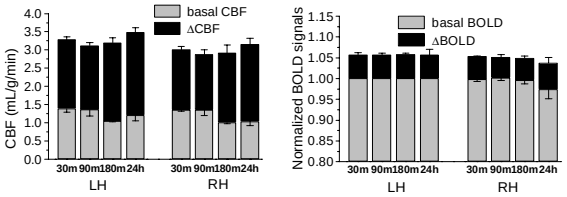

Fig. 2b. Forepaw

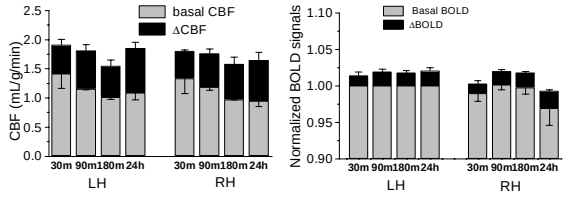

Table 1. Group-average M and $CMRO_2$

		LH	RH
M (%)	30 m	9.2 ± 0.8	9.4 ± 0.4
	90 m	9.4 ± 0.6	8.6 ± 0.5
	180 m	8.2 ± 0.3	7.7 ± 0.4
	24 hr	8.8 ± 2.1	9.1 ± 1.6
Δ $CMRO_2$ (%)	30 m	13 ± 2	13 ± 2
	90 m	19 ± 3	19 ± 3
	180 m	16 ± 3	16 ± 3
	24 hr	21 ± 10	21 ± 10