Negative fMRI response in the striatum: a marker for striatal functional integrity in ischemic rat brain

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INTRODUCTION Stroke is third leading cause of death and long-term disability [1]. Damages to the striatum – which is involved in sensory discrimination, initiation of motor reaction, among others – are known to have severe behavioral impairment [2-4]. Unfortunately, in vivo imaging of functional impairment of the striatum after stroke in animal models remains to be a major challenge due to the lack of non-invasive imaging methodologies that can evaluate striatal function longitudinally with high spatiotemporal resolution. We have recently established an fMRI protocol that can reliably evoke the rat striatal negative CBV fMRI responses for the first time [5-7]. The present study employed this approach to longitudinally investigate the functional integrity in the striatum in stroke rats. Our results demonstrated that the negative striatal fMRI response reliably depicts striatal functional recovery over time and serves as a novel imaging marker other than ADC and CBF. Longitudinal evaluation of subcortical functional recovery and treatment efficacy in anesthetized rat stroke model is possible

METHODS Monocrystalline iron oxide nanoparticle (MION, 30 mg Fe/kg, i.v.) CBV fMRI was performed on two stroke rats under 1.1-1.2% isoflurane and mechanical ventilation. Ischemia was induced by intraluminal middle cerebral artery occlusion for 45 mins followed by reperfusion [8]. MRI was performed longitudinally on *Day 1* and *Day 7* on Bruker 7T Biospec with a surface coil (ID~2cm). ADC and CBF were acquired as described previously [9]. fMRI data were acquired with single-shot GE EPI, with spectral width = 300 kHz, TR/TE = 1000/13.8 ms, FOV = 2.56x2.56 cm, slice thickness = 1.5 mm, and matrix = 96x96. Striatal and cortical responses were evoked by bilateral forepaw electrical stimuli with 10 mA to generate stronger striatal vasoconstriction [4-6], 12 Hz to provide optimal hemodynamic response in isoflurane anesthetized rats [10], and 3 ms pulse duration which has been optimized in our pilot studies. Stimulation paradigm was 60s OFF, 30s ON, and 60s OFF. Five to ten repeated trials were made on each measurement. Data analysis was performed using a custom-built image processing interface [11].

RESULT This study demonstrated a novel fMRI application to investigate the striatal functional integrity after transient focal ischemia. Striatal dysfunction was successfully depicted in stroke rats (**Fig 1**). **Fig 1A** shows a rat with marked lesion in ADC and CBF after reperfusion. The striatal and cortical responses at the right hemisphere were impaired up to Day 7. **Fig 1B** shows a rat with small lesion in ADC and mostly recovered CBF in the striatum after reperfusion. The CBV fMRI, however, showed no response. This indicates that the striatal negative fMRI signal provides additional functional information other than ADC and CBF. On Day 7, the striatal response showed partial recovery at the ventral part (**Fig 1B**).

DISCUSSION The striatum involves in various aspects of brain signaling. Stroke in the striatum has been implicated in many subsequent neurological disorders, such as Alzheimer's disease [2] and Parkinson's disease [3]. Patients and animals with striatal ischemia are also known to have late-onset cognitive and behavioral impairment [4]. Our recent studies showed noxious forepaw electrical stimulation surprisingly evoked sustained negative CBV fMRI responses in the bilateral striatum [5,6] whereas the local neuronal spike and c-Fos activities increased [6]. Such negative fMRI signals in the striatum were markedly attenuated by intravenous injection of a dopamine D_2/D_3 receptor antagonist [6] or lesion of a major dopaminergic afferent pathway in the substantia nigra by 6-hydroxydopamine [7]. These findings were attributed to the effect of dopamine action which is known to have vascular effect, in which stimulating the D_2/D_3 receptors causes vasoconstriction [12,13]. Prior to our studies, there was lack of animal fMRI protocol to evoke the striatal response in stroke rats. Our findings complement the existing stroke fMRI procedures [14-16] and making it possible to investigate the functional reorganization and treatment efficacy in the striatum of the same rat over time. Future studies will investigate striatal and cortical functional responses at chronic stage (Day 28), compare different MCAO durations, and use early striatal fMRI response to predict the apomorphine-induced rotation behavior at 35 days after MCAO.

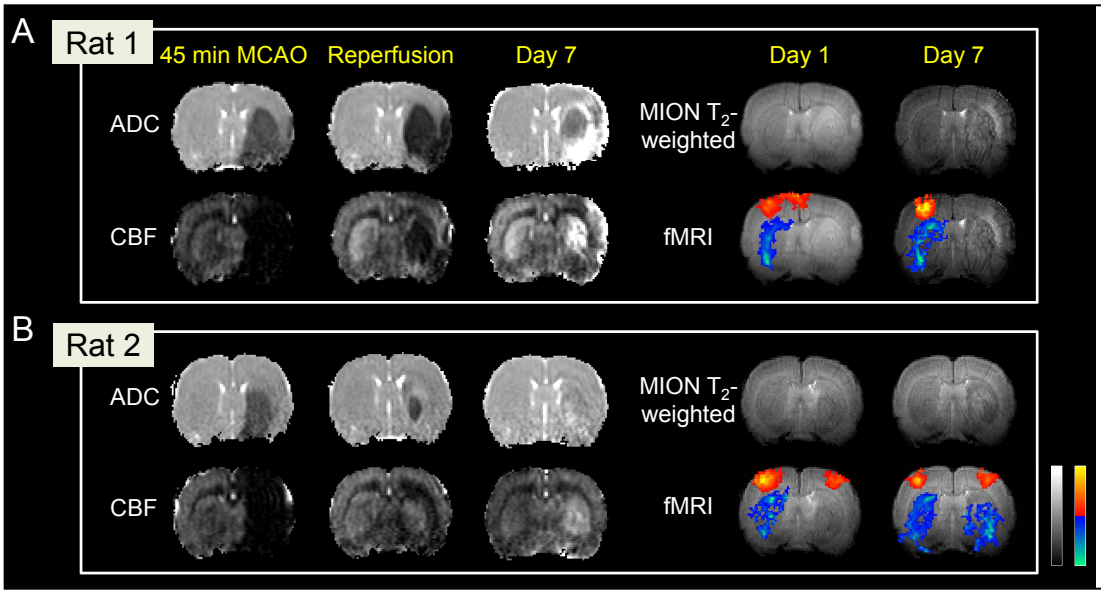

Fig 1. Longitudinal ADC, CBF, MION T_2-weighted, and CBV fMRI of two stroke rats. Both rats underwent 45 min MCAO followed by reperfusion. **(A)** In Rat 1, CBV fMRI responses on Day 1 were only evident in the cortex and striatum contralateral to the lesioned side. This pattern remains similar up to Day 7, albeit the ipsilateral CBF showed hyperperfusion. **(B)** In Rat 2, better ADC and CBF outcomes were observed after reperfusion. Bilateral cortical with contralateral striatal responses were observed. Ipsilateral striatum had no response, even ADC and CBF showed some recovery. On Day 7, ipsilateral striatal response occurred. Color bars indicate ADC ranging from 0.0002 to 0.001 mm^2/s, CBF from 0 to 2.5 ml/g/min, and CBV fMRI (ΔR_2^*) from 2 to 20 and -2 to -8 s^{-1}.

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