Optimizing negative fMRI response in the rat striatum under isoflurane anesthesia

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INTRODUCTION The striatum receives numerous afferent inputs from different brain regions and acts as an important neurochemical signal relay in the brain [1]. Dysfunctions of the striatum have been implicated in many neurological disorders, including Parkinson's and Huntington's disease [2]. Recent reports showed that unilateral noxious forepaw electrical stimulation in rats surprisingly evoked sustained negative BOLD, CBF, and CBV fMRI responses in the bilateral striatum with no significant difference between the two hemispheres [3-5], whereas the local neuronal spike and c-Fos activities increased under α-chloralose anesthesia [5]. This negative fMRI response is associated with vasoconstrictive neurotransmission [5] and has potential to serve as an imaging marker for assessing striatal functional integrity. The present study aimed to (i) establish an optimal stimulation protocol for the rat striatal responses under isoflurane anesthesia which allows longitudinal studies and (ii) to test this survival protocol in a focal ischemic rat brain. This protocol may have long-term applications for the longitudinal study of striatal functional reorganization and plasticity.

METHODS Monocrystalline iron oxide nanoparticle (MION, 30 mg Fe/kg, i.v.) CBV fMRI was performed on 8 normal rats and 1 stroke rat under 1.1-1.2% isoflurane, mechanical ventilation, and paralysis. MRI was performed on a Bruker 7T Biospec scanner with a surface coil (ID~2cm). Single-shot GE EPI was acquired using spectral width = 300 kHz, TR/TE = 1000/13.8 ms, FOV = 2.56x2.56 cm, slice thickness = 1.5 mm, and matrix = 96x96. Nine forepaw stimulus pulse durations (0.1, 0.3, 0.5, 1, 3, 5, 7, 10, and 30 ms) were studied, with fixed 10 mA amplitude generating optimal striatal vasoconstriction [3-5] and 12 Hz square wave providing optimal BOLD,CBF, and evoke field potentials in isoflurane anesthetized rats [6]. Stimulation paradigm was 60s OFF, 30s ON, and 60s OFF. Ischemia was induced by intraluminal middle cerebral artery occlusion for 45 mins [7]. Data analysis was performed using a custom-built image processing interface [8]. Stimulus-evoked changes were tabulated for the contralateral S1 (cS1), ipsilateral S1 (iS1), and ipsilateral striatum (CPu). Statistical analysis employed ANOVA followed by Fisher's post-hoc test. Significant level was set at $p<0.05$. Error bars were SEM.

RESULT & DISCUSSION This study established a novel fMRI protocol to evoke negative fMRI response in the rat striatum under isoflurane anesthesia. The major findings were: (i) the contralateral S1 positve responses peaked around 3 ms pulse duration, (ii) the bilateral striatal responses were negative for 1-3 ms pulse durations, (iii) the ipsilateral S1 postive responses reached to a plateau at 5 ms pulse duration and remained elevated up to 30 ms duration, (iv) this fMRI protocol successfully depicted striatal dysfunction in a stroke rat and can be used for longitudinal assessment of disease evolution as well as functional recovery/reorganization. The 3 ms peak of the cS1 activation may be due to a larger amount of current delivered to the animals compared with short pulses, but have not yet limited by neurovascular refractory period. Ipsilateral S1 increased exponentially with the pulse duration. The ipsilateral cortical response may be a combination of painful stimulus evoked bilateral cortical activation [8,9] and/or non-specific cardiovascular effect. The later component was evident in the CBV fMRI time courses, showing a sustained post-stimulus elevation in longer pulse durations. The striatal fMRI responses showed an interesting band-pass shape, with 1-3 ms evoked stronger negative fMRI signals. The reduced negative fMRI responses after 5 ms duration may also be attributed to non-specific cardiovascular effect. Future studies will incorporate electrophysiology to investigate the source of striatal negative fMRI signals and the neurovascular coupling mechanism with different stimulation parameters.

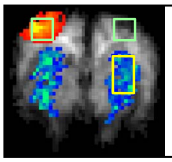

Fig 1. CBV fMRI of unilateral noxious forepaw stimulation under isoflurane anesthesia (10 mA, 12 Hz, and 1 ms pulse duration). Clear vasodilation was observed in the cS1, whereas sustained vasoconstriction was observed in the bilateral striatum.

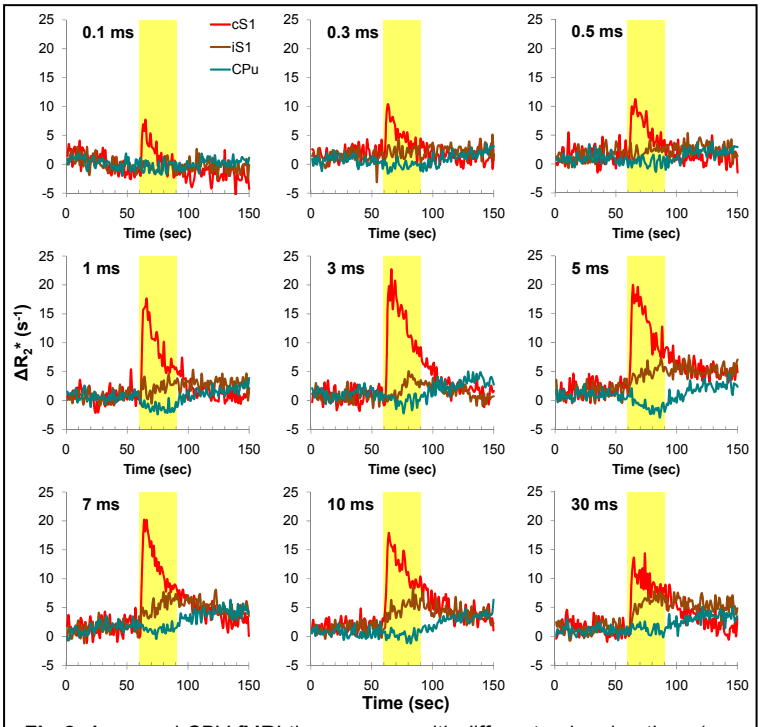

Fig 2. Averaged CBV fMRI time-courses with different pulse durations (n = 8). The amplitude and frequency were fixed at 10 mA and 12 Hz, respectively. ROIs used are shown in Fig 1.

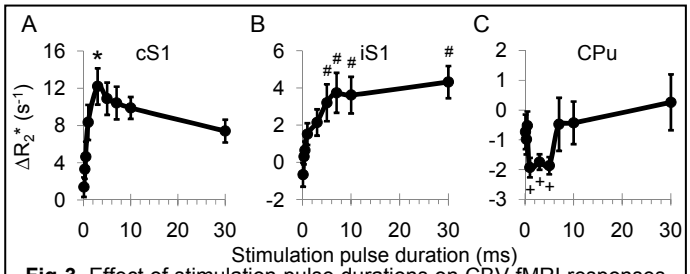

Fig 3. Effect of stimulation pulse durations on CBV fMRI responses (n = 8). **(A)** *3 ms significantly different from 0.1, 0.3, 0.5, and 30 ms. **(B)** #5, 7, 10, and 30 ms significantly different from 0.1, 0.3, and 0.5 ms. **(C)** +1, 3, and 5 ms significantly different from 30 ms.

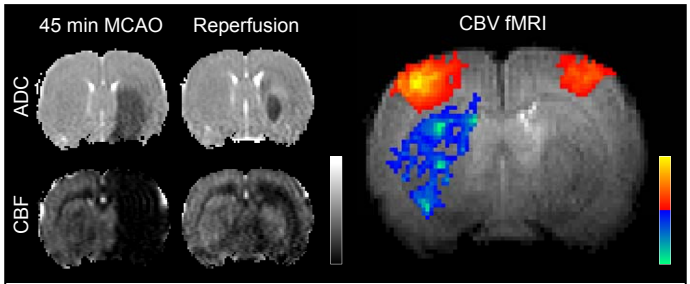

Fig 4. A model of 45 min right middle cerebral artery occlusion followed by reperfusion was used to verify the established protocol. Forepaw stimulation was applied *bilaterally* with 10 mA, 12 Hz, and 3 ms. No negative CBV response was observed in the right striatum, albeit the striatal blood flow was salvaged. The animal was recovered after fMRI, demonstrating this protocol can be used for longitudinal assessment.

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