A modified photothrombotic stroke model using implantable optic fiber: in-bore stroke induction for probing peri-infarct spreading depolarization

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INTRODUCTION Peri-infarct spreading depolarization (PID) is a series of propagating electric waves that appear during the hyperacute phase of stroke [1]. PID has been shown to accelerate the stroke progress [2], and suppressing PID is known to reduce the final infarct size [3]. Optical imaging and EEG/ECoG recording allow identification of PID, but both techniques are depth-limited and cannot provide volumetric tissue information with clinically relevant indices. PID primarily appears within 30 min after stroke onset in rodent models [4]. To our knowledge, PID has never been characterized by MRI due to relatively long setup time and a lack of consecutive imaging data with high temporal resolution. This study aimed to **(i)** develop a highly reproducible model allowing in-bore stroke induction and **(ii)** characterize PID using quantitative perfusion and diffusion MRI. A modified photothrombotic model using miniature implantable optic fibers (IOF photothrombosis) was established, and perfusion/diffusion data were acquired in either an interleaved manner with 2.5 min temporal resolution or sequentially with 3 s temporal resolution. The IOF photothrombotic stroke model proposed by this study can induce an ischemic lesion in any predetermined brain area, which opens up new avenues for preclinical stroke research.

METHODS Fourteen male Sprague Dawley rats (250–300 g) were anesthetized with 1.5% isoflurane, ventilated and paralyzed. A home-made MR-compatible optic fiber (200 μm) was placed above the primary somatosensory cortex of forelimb (S1fl), and an 8 m optic patch cable was used to deliver green-light laser (532 nm, 10 mW) for 8 min. Rose Bengal (10 mg/kg) was intravenously administered 1 min after the laser onset for 2 min. MRI was performed on a Bruker 9.4T Biospec scanner with a home-made surface coil (ID=2 cm) and a separate neck coil for arterial spin labeling (ASL). CBF and ADC were alternatively acquired every 2.5 min for 6 h including 40 min baseline before stroke induction (n=6). CBF was measured by continuous ASL using single shot gradient-echo EPI with bandwidth=250 kHz, TR/TE=3000/12 ms, labeling duration = 2.4 s and post-labeling delay=250 ms, matrix=96x96, FOV=2.56x2.56 cm^2, and slice thickness=1 mm. ADC was acquired with the same geometry using single shot spin-echo EPI with bandwidth=250 kHz, TR/TE=3000/30 ms, number of A0=1, number of directions=3 and b-value=1200 s/mm^2. In the second group of rats, CBF or ADC were acquired continuously for 30 min (n=2). In the third group of rats, optic fibers were implanted into the somatosensory cortex, nucleus accumbens and subthalamic nucleus (n=3). In the fourth group of rats, fibers were implanted above the S1fl and different Rose Bengal dose and laser duration were tested (n=3). T_2-weighted images were acquired 24 h after stroke. Image data were processed similarly as described elsewhere [5]. Statistical analysis was performed by ANOVA with Fisher's post-hoc test and paired t-tests. The number of PID was tabulated. Significant level was set at $P<0.05$. Error bars were SEM.

RESULT & DISCUSSION This study demonstrates an animal model capable of producing a localized stroke inside the MRI while allowing visualization of CBF and ADC change during the stroke onset. After laser illumination, the ADC and CBF in the target area dropped 30 and 50% within 10 min, respectively, and then continually declined over the next 5 h. The CBF reached a steady level at 120 min ($P<0.05$), faster than the ADC which did not become stable until 210 min ($P<0.05$) (**Fig. A&E**). Within the first 30 min after stroke onset, an average of 3.7±2.31 (mean±SD) PIDs was observed. PID in our experimental condition was described by either a decrease in ADC due to propagating cell swelling as reported by *ex vivo* immunofluorescence technique [6], or spreading-hyperemia, in which CBF rises more than 100% due to energy demand to restore homeostasis as observed by open-skull optical imaging [7]. The PIDs initiated around the ischemic core region [2,4,7], propagated outward, and then vanished when reaching the corpus callosum and the brain midline, and were confined to the ipsilesional cortex. We demonstrated for the first time that post-PID oligemia [1,8] is not restricted to the PID territory but occurs on a whole-brain scale (**Fig. E&H**). The ischemic location in the proposed IOF photothrombotic model can be targeted by implanting the fiber into predetermined brain areas (**Fig. J**) and the infarct size can be controlled by the Rose Bengal dose and the laser duration (**Fig. K**). This is in sharp contrast to the most widely used middle cerebral artery occlusion (MCAO) model which is only capable of creating an ischemic lesion in the MCA territory with relatively low reproducibility [9]. Our model may prove useful to investigate PID and has potential to systematically study neuropathological outcome of stroke at brain regions that were previously inaccessible. Future studies will attempt to assess functional plasticity changes in the chronic phase of stroke with different tPA treatment delay.

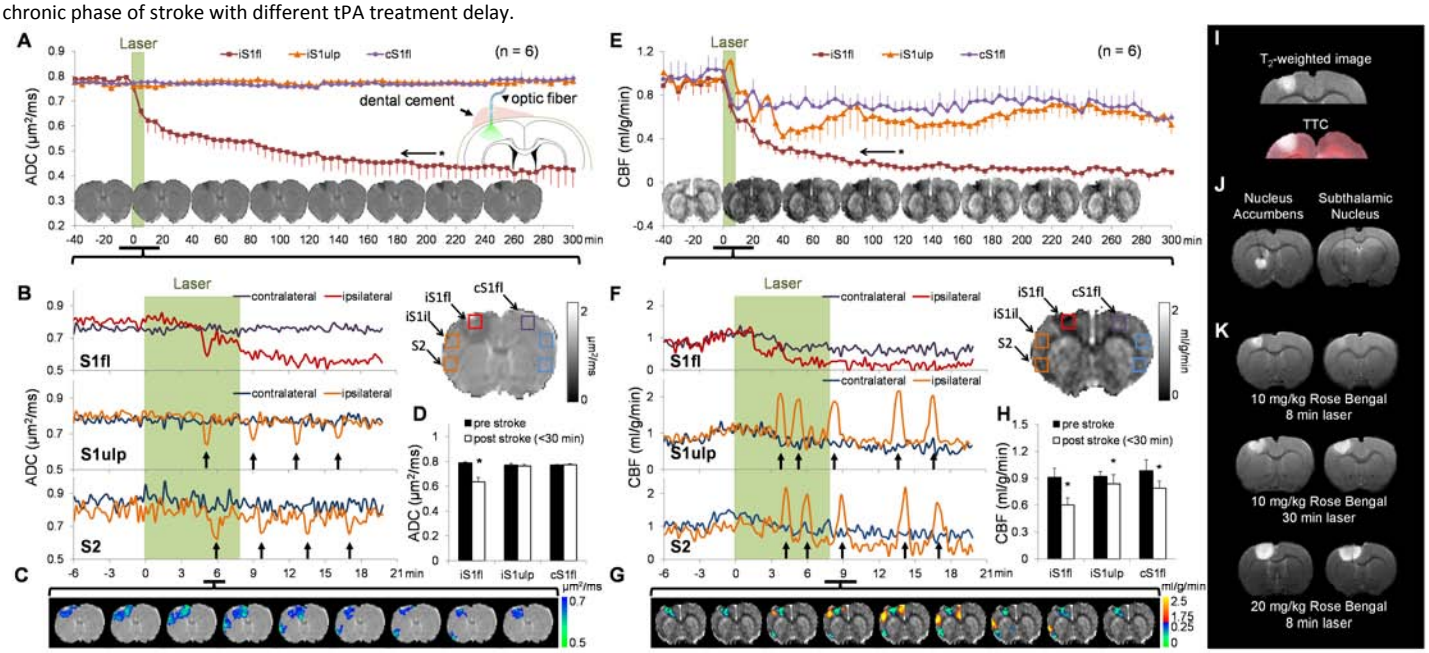

Figure (A) Interleaved ADC data were acquired every other 2.5 min (n=6). The inset is a schematic plot of the proposed model. Susceptibility artifact was reduced by adding dental cement and toothpaste. The inset time-series images on the horizontal axis are corresponding ADC maps every 40 min. **(B)** Continuous ADC data were acquired every 12 s during the stroke onset. ROIs are shown in inset. PIDs can be clearly identified in the time courses (black arrows) with a propagated speed of ~2 mm/min. **(C)** Color-coded time series ADC images during a single PID session with 12 s resolution. **(D)** Group-averaged ADC changes before and after stroke onset (n=6). *indicates significant difference from pre-stroke values. **(E-H)** Corresponding CBF data. Data presentation is identical, except the temporal resolution of the CBF data was 3 s in **(F)** and 18 s in **(G)**. **(I)** Ischemic lesion was confirmed with T_2-weighted image and TTC stain in the same subject. **(J)** Optic fiber implanted into deep brain areas to create ischemic lesions. **(K)** Lesion size controlled by manipulating the Rose Bengal dose or laser exposure duration with identical fiber implantation.

REFERENCE [1] Dreier, Nat Med, 2011, 17:439. [2] Dohmen et al., Stroke, 2008, 63:720. [3] Shin et al., Brain, 2007, 130:1631. [4] Nakamura et al., Brain, 2010, 133:1994. [5] Shih et al., JCBFM, 2011, 31:832. [6] Takano et al., Nature, 2007, 10:754. [7] Ayata et al., JCBFM, 2004, 24:1172. [8] Lauritzen, Brain, 1994, 117:199. [9] Chen et al., Animal models of acute neurological injuries. 2009.