

ORIGINAL ARTICLE

Imaging neurovascular function and functional recovery after stroke in the rat striatum using forepaw stimulation

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Negative functional magnetic resonance imaging (fMRI) response in the striatum has been observed in several studies during peripheral sensory stimulation, but its relationship between local field potential (LFP) remains to be elucidated. We performed cerebral blood volume (CBV) fMRI and LFP recordings in normal rats during graded noxious forepaw stimulation at nine stimulus pulse widths. Albeit high LFP–CBV correlation was found in the ipsilateral and contralateral sensory cortices ($r = 0.89$ and 0.95 , respectively), the striatal CBV responses were neither positively, nor negatively correlated with LFP ($r = 0.04$), demonstrating that the negative striatal CBV response is not originated from net regional inhibition. To further identify whether this negative CBV response can serve as a marker for striatal functional recovery, two groups of rats ($n = 5$ each) underwent 20- and 45-minute middle cerebral artery occlusion (MCAO) were studied. No CBV response was found in the ipsilateral striatum in both groups immediately after stroke. Improved striatal CBV response was observed on day 28 in the 20-minute MCAO group compared with the 45-minute MCAO group ($P < 0.05$). This study shows that fMRI signals could differ significantly from LFP and that the observed negative CBV response has potential to serve as a marker for striatal functional integrity in rats.

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INTRODUCTION

The striatum receives afferent inputs from cerebral cortex, ventromedial, ventrolateral, and mediodorsal thalamic nuclei and projects major efferent outputs to globus pallidus interna/externa and substantia nigra pars reticulata.¹ It plays a pivotal role in several neurodegenerative disorders and regulating motor behavior. Recent reports showed that unilateral noxious forepaw electrical stimulation in rats evoked sustained negative blood oxygenation level dependent (BOLD), cerebral blood flow (CBF), and cerebral blood volume (CBV) functional magnetic resonance imaging (fMRI) responses in the bilateral striatum with no significant difference between two hemispheres,^{2–6} while the neuronal spike and c-Fos activities increased.⁶ This negative response has also been observed in epileptic rats during whisker stimulation⁷ or normal rats during direct nerve stimulation.⁸ Functional magnetic resonance imaging response has been shown to correlate better with local field potential (LFP) instead of spike;⁹ however, whether this particular negative fMRI response matches with LFP changes remains unclear.

Nociceptive stimuli have been shown to reliably evoke this intriguing negative fMRI responses in the rat striatum under alpha-chloralose anesthesia,^{2,5,6,10} medetomidine sedation,⁴ or medetomidine combined with light isoflurane (0.4%) anesthesia.³ This

stimulation model has potential to serve as a functional imaging marker for striatal functional integrity for various types of preclinical applications in rodents. Nevertheless, a stable model for longitudinal imaging of striatal function is missing. Alpha-chloralose is not an ideal anesthetic for survival study because of its gastrointestinal inflammatory effect,¹¹ while the sedative effect of medetomidine is time dependent and is not ideal for studies with longer experimental time.¹² In contrast, soflurane is the most widely used anesthetics for small animal imaging. Robust and repeatable fMRI responses have been shown in the primary somatosensory cortex (S1) in well-ventilated rats under isoflurane anesthesia.^{13–15} Whether the aforementioned negative striatal fMRI response can be observed under isoflurane anesthesia remain unknown. The ability to do so could have long-term applications to assess striatal function during disease progression as well as monitor treatment effects *in vivo*.

To these ends, we used a well-established CBV fMRI technique in mechanically ventilated rats under isoflurane anesthesia. Nine forepaw stimulus pulse widths (0.1, 0.3, 0.5, 1, 3, 5, 7, 10, and 30 milliseconds) were studied, with fixed 10 mA amplitude known to generate optimal CBV decreases^{2,4,6} and 12 Hz stimuli known to evoke robust CBF and LFP responses in the primary somatosensory cortex (S1) under 1% to 1.2% isoflurane anesthesia.^{13,14,16}

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Local field potentials were recorded at the ipsilateral S1 (iS1), contralateral S1 (cS1), and ipsilateral striatum (iCPu) in separate animals under identical experimental conditions to corroborate the fMRI findings. Contralateral CPu (cCPu) response was not recorded because of our hardware limitation and the fact that no difference was detected between the two hemispheres in this model.^{2–6} To investigate whether the striatal negative fMRI response can serve as a marker for striatal functional integrity, we used stimulation of 10 mA, 3 milliseconds pulse width, and 12 Hz in two groups of rats underwent 20- and 45-minute middle cerebral artery occlusion (MCAO) and followed the fMRI responses up to 28 days.

MATERIALS AND METHODS

Subjects

A total of 33 adult male Sprague-Dawley rats (weighing 250 to 300 g, 8 to 10 weeks old; Charles River Laboratories) were studied. All experimental procedures were approved by the Institutional Animal Care and Utilization Committee, UT Health Science Center at San Antonio and were performed under ARRIVE guidelines. Animals were housed in the vivarium (12:12-hour light–dark cycle, controlled humidity and temperature) with free access to food and water. In the first study (imaging striatal function), 11 normal rats were used to determine the optimal CBV fMRI responses with varied pulse widths under isoflurane anesthesia and 5 normal rats were used to investigate the corresponding LFP responses under the same experimental conditions. In the second study (imaging striatal functional recovery), two groups of 5 rats each were included to longitudinally study the striatal function and functional recovery with different occlusion time before and after stroke up to 28 days. Of note, 3 additional rats in the second study died before the last imaging time point (day 28) and were excluded from the sample size pool. In addition, a group of 4 rats were used to verify whether the striatal functional recovery has a neural origin and an additional group of 5 rats were used to study the effect of varying pulse width on mean arterial blood pressure (MABP) and heart rate.

Animal Preparation

Rats were initially anesthetized with 3% isoflurane and orotracheally intubated for mechanical ventilation (Model 683, Harvard Apparatus, South Natick, MA, USA). After the animal was secured in an MRI-compatible rat stereotaxic headset, isoflurane was reduced to 1% to 1.2%. End-tidal CO₂ was continuously monitored via a capnometer (Surgivet, Smith Medical, Waukesha, WI, USA) and kept between 3% and 3.5%. Noninvasive end-tidal CO₂ values were calibrated against invasive blood-gas samplings under identical baseline conditions, resulting an arterial pCO₂ of 37.6 ± 4.7 mm Hg (mean ± s.d., *n* = 8).¹⁷ Rectal temperature was maintained at 37.0°C ± 0.5°C with warm-water circulating pad. Heart rate and blood oxygen saturation level were continuously monitored by a MouseOx system (STARR Life Science Corp., Oakmont, PA, USA). The heart rate was kept between 350 and 400 bpm and oxygen saturation level was maintained above 97%. An established CBV fMRI technique was employed¹⁸ by using high-dose 30-mg/kg monocrySTALLINE iron oxide nanoparticles to eliminate potential BOLD contamination.

Stimulation

In the first study, two needle electrodes were inserted under the skin of the right forepaw: one between the first and second digits and the other between the third and fourth digits. These electrodes were then fixed with surgical tape and the stimulation was confirmed by observing digit twitching. Nine forepaw stimulus pulse widths (0.1, 0.3, 0.5, 1, 3, 5, 7, 10, and 30 milliseconds) were studied in a pseudo-random manner, with fixed 10 mA amplitude generating optimal CBV decreases^{2,4,5} and 12-Hz square wave providing robust CBF and LFP responses under 1% to 1.2% isoflurane anesthesia.^{13,14,16} This stimulation amplitude is known to induce pain and therefore striatal responses.^{3,6} Strong digit and forelimb twitching was observed during noxious stimulation. However, no or noticeable motion artifact was detected. In addition, the subjects showed no physical damage in the ear canal at the end of the experiment, indicating the animal head was secured and stabilized by the ear bars during imaging, and the anesthesia level used in this study should be sufficient to suppress major motion. Two to five repeated trials were made for each pulse width. In the second study (imaging striatal functional recovery), needle electrodes were

inserted under the skin of the left and right forepaw, such that both forepaws were stimulated simultaneously in series at 10 mA, 12 Hz, and 3 milliseconds pulse width. To evaluate the effect of varying stimulus pulse width, MABP and heart rate were measured via a PE-50 tubing cannulated to femoral artery. For all stimulations, at least 3 minutes break was given between trials.

Magnetic Resonance Imaging Experiments

In the first study (imaging striatal function) on normal rats (*n* = 11), MR images were acquired on a 7T, 30-cm bore magnet and a 40 G/cm gradient (Bruker, Billerica, MA, USA). A custom-made circular surface coil (ID ~2 cm) was placed on the rat head. Magnetic field homogeneity was optimized using standard FASTMAP shimming with first-order shims on an isotropic voxel of 7 × 7 × 7 mm encompassing the imaging slices. A T₂-weighted pilot image was taken in the midsagittal plane to localize the anatomic position by identifying the anterior commissure (bregma –0.8 mm).¹⁹ Cerebral blood volume fMRI was acquired with single-shot gradient echo-planar imaging sequence using spectral width = 300 kHz, repetition time/echo time = 1,000/13.8 milliseconds, partial Fourier acquisition = 5/8, field of view = 2.56 × 2.56 cm², slice thickness = 1.5 mm, number of slice = 7, and matrix = 96 × 96. Functional magnetic resonance imaging paradigm included 60 sets of echo-planar images before stimulation, 30 sets during stimulation, and 60 sets after stimulation.

In the second study (imaging striatal functional recovery), rats were subjected to 20-minute (*n* = 5) and 45-minute (*n* = 5) MCAO. Reperfusion was performed by withdrawing the occluder into the external carotid artery, restoring blood flow to the brain. To achieve 20-minute MCAO, we unfortunately could not perform CBF MRI before reperfusion. For the 20-minute MCAO group, MR images were longitudinally acquired at control time point, (3 to 5 days before MCAO), on day 0 (immediately after MCAO and reperfusion), day 7, and day 28 after MCAO (total of 20 MRI experiments). Imaging time points were essentially the same for the 45-minute MCAO group except the control (pre-MCAO) data were not acquired (total of 15 MRI experiments).

In addition to the fMRI, quantitative CBF and apparent diffusion coefficient (ADC) were measured before monocrySTALLINE iron oxide nanoparticle injection and fMRI runs. CBF was acquired by continuous arterial spin-labeling technique with a separate neck coil for labeling using single-shot, gradient echo-planar imaging, spectral width = 300 kHz, repetition time/echo time = 3,000/10.3 milliseconds, partial Fourier acquisition = 5/8, labeling duration = 2.46 seconds, postlabeling delay = 0.25 seconds, field of view = 2.56 × 2.56 cm², slice thickness = 1.5 mm, and acquisition matrix = 96 × 96. Sixty pairs of images were acquired for signal averaging. Diffusion-weighted images were acquired with single-shot, spin-echo, echo-planar imaging along 30 diffusion encoding directions with *b* value = 0 and 1.2 milliseconds/μm² with same localization and resolution as CBF. Other imaging parameters were: δ/Δ = 4/16 milliseconds, repetition time = 3,000 milliseconds, and echo time = 35 milliseconds. Acquisitions were repeated four times to increase signal-to-noise ratio.

Electrophysiology Recording

In the first study (imaging striatal function), electrophysiology was performed in a separate group of rats (*n* = 5). The procedure was essentially the same as that described previously.²⁰ A stereotaxic frame (Model 900, David Kopf Instruments, Tujunga, CA, USA) was used to position the head. The animal physiologic conditions were kept the same as the MRI experiment, except craniotomies were performed to implant recording electrodes. A stainless steel screw was placed 2-mm caudal to lambda as a reference electrode. After the dura was removed, custom designed polyimide-based 16-channel microelectrode arrays²¹ with inter-contact spacing of 74 μm were implanted to cS1, iS1 (coordinates from bregma: anterior-posterior +0.5 mm; medial-lateral ±4 mm; and ventral-dorsal –2 mm; approached with a coronal angle of 22.5°), and CPu (anterior-posterior –0.8 mm; medial-lateral +4 mm; and ventral-dorsal –6.5 mm). Data were sampled from the contact lead located at cortical layer V (~VD –1.4 mm) or the dorsal striatum (~VD –5.9 mm), and filtered on a preamplifier between 0.3 and 500 Hz and sampled at 1 kHz. Data were acquired using a Cerebus multichannel data acquisition system (Blackrock Microsystems, Salt Lake City, UT, USA). At the end of the experiment, a 30-μA direct current was delivered to the deepest contact lead for 10 seconds to label the recording site. T₂-weighted MRI scans were performed to confirm the recording site. This method is comparable to histology as it is sufficient to confirm the electrode tip in the target area. In

the second study (imaging striatal functional recovery), LFP was measured in a separate group of rats ($n = 4$), underwent 20-minute MCAO to verify whether neuronal recovery can be observed. The recording parameters were identical to the first study, but with only one electrode implanted into the iCPu and stimulation of 10 mA, 12 Hz, and 3-millisecond pulse width was used. Recording was performed longitudinally at four time points identical to fMRI studies (control, day 0, day 7, and day 28).

Data Analysis

Image analysis was performed using a custom-written program²² in Matlab (Math-Works, Natick, MA, USA) and statistical parametric mapping. Automatic coregistration using statistical parametric mapping codes were applied to realign time-series data to the first time point within subjects using and then again across subjects. Correlation coefficient analysis was performed on a pixel-by-pixel basis to correlate MR signal changes with electrical stimulation paradigm with a significant threshold of $P < 0.05$ (Bonferroni corrected). Spatial averaging across subjects was performed to generate averaged correlation coefficient map after atlas-based coregistration as described previously,²² where CBV increases and decreases are indicated by red–yellow and blue–green colors, respectively. The stimulus-evoked ΔR_2^* value, which varies linearly with the stimulus-evoked CBV fraction, was calculated as follows after monocrySTALLINE iron oxide nanoparticles injection: $\Delta R_2^* = \frac{1}{T_2} \ln(S_{\text{ctrl}}/S_{\text{stim}})$, where S_{ctrl} and S_{stim} are the MR signal intensities before and during stimulation, respectively. CBF was calculated as: $\text{CBF} = \frac{\lambda}{T_1} \frac{S_C - S_L}{S_C + (2\alpha - 1)S_L}$, where S_C and S_L are the MR signal intensities from the control and labeled images, respectively; λ is the water brain–blood partition coefficient, T_1 is that of tissue, and α is the arterial spin-labeling efficiency. The values of λ , T_1 , and α were 0.9, 1.8 seconds, and 0.7, respectively. Apparent diffusion coefficient maps were calculated by using: $\text{ADC} = \ln(S_0/S_1)/(b_1 - b_0)$, where S_1 is the signal intensity obtained with b_1 (1.2 milliseconds/ μm^2) and S_0 is the signal intensity obtained with b_0 (0 milliseconds/ μm^2). Regions of interest (ROIs) were placed on the cS1, iS1 (both 8×8 pixels) and the iCPu (13×8 pixels) to extract fMRI time-course data after the images are coregistered. No apparent spatial shift was observed at different stimulus pulse widths. Therefore data were analyzed with the same ROIs across trials. Slice at bregma -0.8 mm was selected for subsequent analysis because this is the primary area being affected by MCAO and strong cortical and striatal forepaw fMRI responses were also observed at this location.

Electrophysiology data were analyzed by using a custom-written program in Matlab. The 30-second raw LFP data were split into 360 sweeps of 83 milliseconds poststimulus periods based on the stimulus time stamps (12 Hz). The averaged evoked LFP was summated during the 83 milliseconds poststimulus period, denoted as ΣLFP . Data were then averaged across subjects to provide group-averaged responses.

Statistical analysis was performed by SPSS software (IBM, Armonk, NY, USA). Paired t -test was used to compare the responses between hemispheres. Repeated-measures analysis of variance with Fisher's *post hoc* test was used to compare stimulus-evoked changes in ΔR_2^* and ΣLFP values in the first study, and the ΔR_2^* , ADC, CBF, and ΣLFP values at different time points in the second study. The significance level was set at $P < 0.05$.

Ethics Statement

All studies reported followed the guidelines of the OLAW (Office of Laboratory Animal Welfare) Guide for the Care and Use of Laboratory Animals as well as the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines. The experiments were approved by the University of Texas Health Science Center IACUC. The manuscript was written in accordance with the ARRIVE guidelines.

RESULTS

Striatal and Cortical Neurovascular Function in Normal Subjects

Table 1 shows the effect of graded forepaw stimulation on MABP and heart rate. Representative CBV fMRI images at bregma $+0.7$ and -0.8 mm are shown in Figure 1A. Stimulation at right forepaw with 10 mA, 12 Hz, and 3 milliseconds pulse width resulted in significant CBV increases in the cS1 and CBV decreases in bilateral CPu. Figure 1B shows group-averaged functional CBV time-course data under nine different pulse widths (0.1 to 30 milliseconds; $n = 11$). Our previous and current findings showed no significant side-to-side difference of striatal signals between

Table 1. Effect of stimulus pulse width on blood pressure and heart rate (mean \pm s.e.m.)

Pulse width	MABP (mm Hg)		HR (beats per minute)	
	Baseline	Stimulation	Baseline	Stimulation
0.1	122.3 \pm 3.8	125.4 \pm 3.2	406 \pm 6.8	408.7 \pm 6.1
0.3	118.2 \pm 3.6	122.8 \pm 5.1	409.6 \pm 7.6	418.7 \pm 9.6
0.5	120.6 \pm 5.1	129.3 \pm 4.3	417.9 \pm 6.4	424.7 \pm 7.3
1	116.4 \pm 5.4	128.4 \pm 3.4	416.5 \pm 7.4	432.4 \pm 8.6
3	116.4 \pm 5.9	129.6 \pm 4.2	406.4 \pm 10.3	423.6 \pm 11.9
5	120.7 \pm 5.3	137.4 \pm 2.5 ^a	407.2 \pm 10.1	426.6 \pm 13.4
7	119.4 \pm 4.4	134.6 \pm 1.8 ^a	414.5 \pm 8.7	434.1 \pm 9.1
10	117 \pm 4.6	134.9 \pm 3.3 ^a	418.5 \pm 6.3	437.7 \pm 8.8
30	122.6 \pm 4.4	136.7 \pm 8.9 ^a	409.4 \pm 9.9	439 \pm 14.6

Abbreviations: MABP, mean arterial blood pressure; HR, heart rate.
^adenotes $P < 0.05$.

both hemispheres under this stimulation model and therefore the CPu time-course data were only discussed on the ipsilateral side. Cerebral blood volume in the cS1 responded immediately to the stimulation, showing an overshoot at the stimulus onset across all stimulation conditions that gradually decreased over time. In contrast, CBV in the CPu responded comparatively slower to the stimulation and was only apparent at 1 to 5 milliseconds pulses.

Under identical experimental condition, LFPs were recorded at the cS1, iS1, and CPu (Figure 2A). Typical LFP waveforms are shown in Figure 2B. In the cS1, LFP peaked at 50 milliseconds after stimulus onset. This early negative peak was also observed in the iS1 and CPu. Around 100 milliseconds after stimuli onset, a late negative peak was also recorded in the iS1 as well as in the CPu with further delay.²³ For all the nine pulse widths tested in this study, averaged ΣLFP time courses showed a clear overshoot at the stimulus onset and decreased over time in the cS1, iS1, and CPu (Figure 2C). Increased ΣLFP s were observed in the CPu, which does not match to the trend of CBV changes.

Figure 3A compared CBV fMRI and ΣLFP data in cS1, iS1, and CPu under nine stimulation pulse widths. In the cS1, both CBV and ΣLFP increased with pulse width ($P < 0.05$), reaching a peak around 3 to 10 milliseconds, and then decreased at 30 milliseconds pulses ($P < 0.05$). In the iS1, both CBV and ΣLFP increased with stimulus pulse width and reaching a plateau ~ 3 to 5 milliseconds ($P < 0.05$). The peak CBV and ΣLFP responses were ~ 3 and 5 times lower compared with the responses in cS1, respectively. In the CPu, CBV responses were mostly negative and peaked ~ 1 to 3 milliseconds ($P < 0.05$), while the ΣLFP responses were mostly positive and peaked ~ 5 to 10 milliseconds ($P < 0.05$), similar to that in the cS1. Significant correlation was found between CBV and ΣLFP in the cS1 ($r = 0.89$, $P < 0.05$) and the iS1 ($r = 0.95$, $P < 0.05$), but not in the CPu ($r = 0.04$, $P > 0.05$; Figure 3B).

Striatal and Cortical Apparent Diffusion Coefficient and Cerebral Blood Flow Changes after Stroke

The group-averaged ADC and CBF maps in the 20- and 45-minute MCAO groups ($n = 5$ each) are shown in Figure 4 and their temporal evolution were analyzed for the cortical and striatal ROIs as shown in Figure 6.

In the 20-minute MCAO group, reperfusion restored ADC values in the ROIs and showed no significant difference compared with the contralateral hemisphere on day 0 ($P > 0.05$). This may be because the ischemic lesion core was only partially included in the iCPu ROI and primarily located at the external globus pallidus. Ipsilateral striatum showed higher ADC on day 7 and day 28 ($P < 0.05$). Cerebral blood flow values in the iS1 and iCPu decreased significantly on day 0 compared with the contralateral

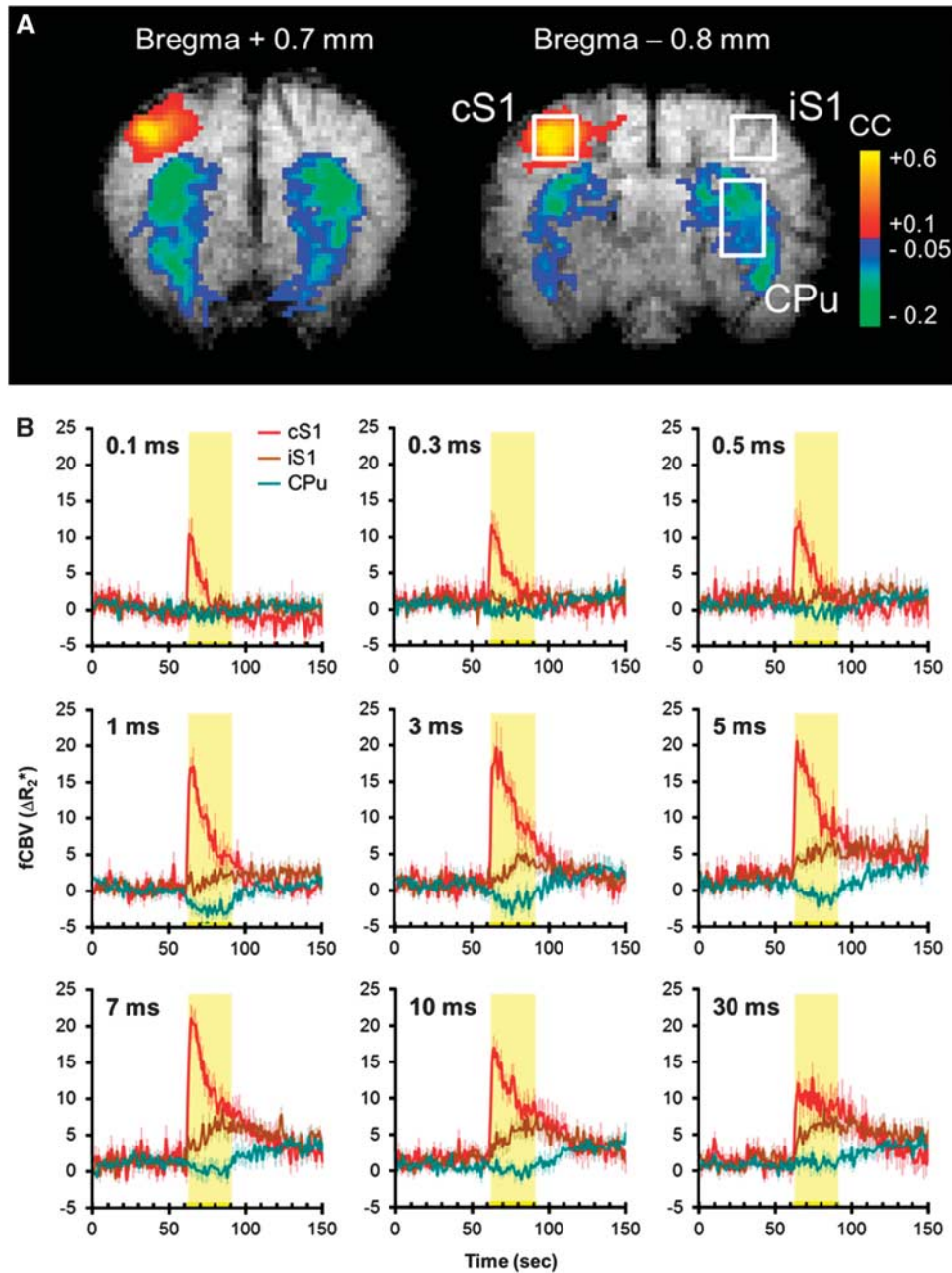


Figure 1. Cerebral blood volume functional magnetic resonance imaging (CBV fMRI) of right forepaw stimulation under isoflurane anesthesia. **(A)** Representative fMRI responses at two adjacent slices. Stimulation was 10 mA, 12 Hz, and 3 milliseconds. Stimulation-evoked CBV elevation was evident in the contralateral S1 (cS1), whereas CBV reduction was evident in the CPU of both hemispheres. **(B)** Group-averaged CBV fMRI time courses in the cS1, ipsilateral S1 (iS1), and ipsilateral striatum (CPU; $n = 11$). Regions of interest are shown in **A**. The yellow-shaded regions indicate stimulation period. Stimuli fixed at 10 mA and 12 Hz. The intensity of the time-course data (ΔR_2^*) is linearly related to CBV fraction, in a unit of per second. Error bars are s.e.m. values.

side and the prestroke control ($P < 0.05$). CBF in the iS1 increased on day 7 compared with day 0 ($P < 0.05$), but was still significantly lower than cS1 ($P < 0.05$). Cerebral blood flow in the iCPU increased on day 7 compared with day 0 ($P < 0.05$) and was significantly higher than cCPU ($P < 0.05$). On day 28, CBF in the iCPU was recovered and was not significantly different from cCPU ($P > 0.05$), while the CBF in the iS1 was still significantly lower than the prestroke control and the cS1 ($P < 0.05$).

In the 45-minute MCAO group, significant ADC decrease in the iS1 was found immediately after reperfusion on day 0 ($P > 0.05$). Similar to the 20-minute MCAO group, iCPU also showed a slightly

lower ADC compared with control, but was not significantly different from the cCPU ($P > 0.05$). Ipsilateral striatum showed higher ADC on day 7 and day 28 ($P < 0.05$). On day 28, the ADC was significantly higher than that in the 20-minute MCAO group ($P < 0.05$). Cerebral blood flow values in both the iS1 and iCPU were significantly lower on day 0 compared with the contralateral side ($P < 0.05$). On day 7, significant hyper-perfusion was found in the iCPU compared with day 0 as well as to the matched time point in the 20-minute MCAO group ($P < 0.05$). Hyper-perfusion in the cortex was apparent on the lateral side of the brain and the boundary of the hyper-perfused area

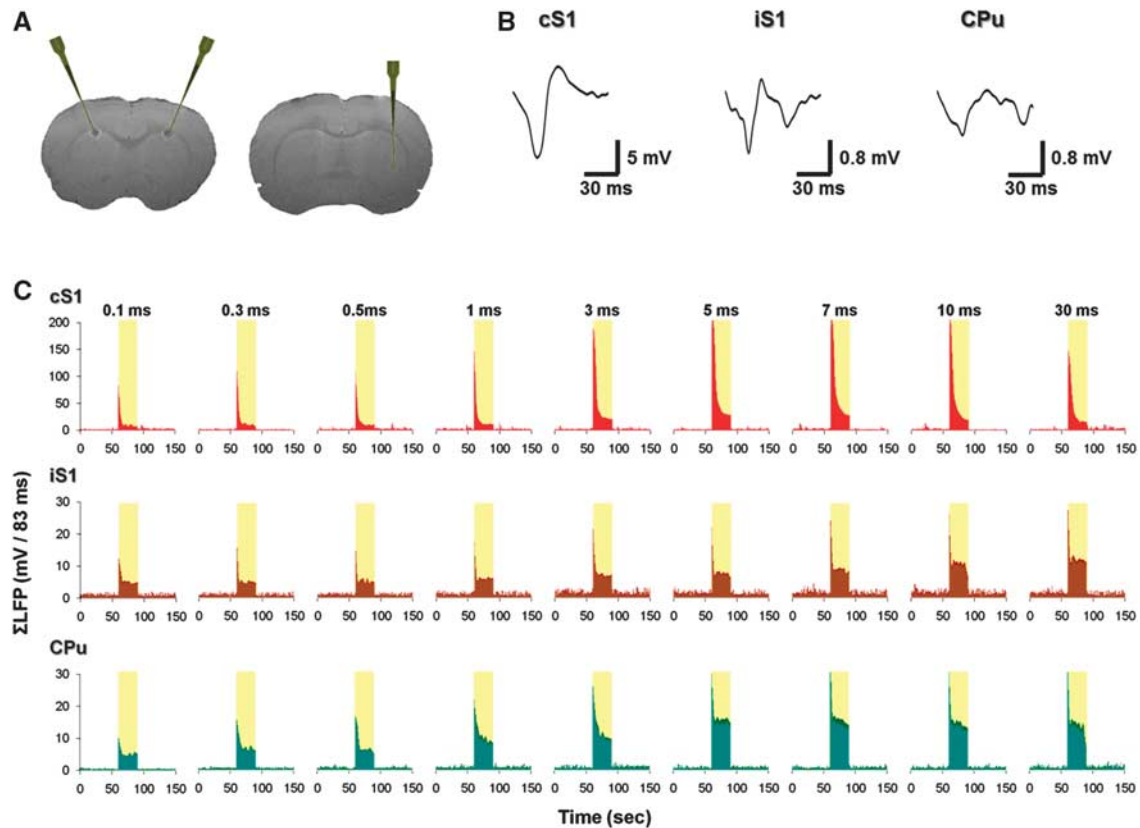


Figure 2. Local field potential (LFP) recording of right forepaw stimulation under isoflurane anesthesia. (A) T_2 -weighted magnetic resonance imaging scans showing the recording site. At the end of the experiment, a $30\text{-}\mu\text{A}$ direct current was delivered to the deepest contact lead for 10 seconds for each electrode. Data were recorded at cortical layer V, ~ 0.6 mm above the lesion site. (B) Typical averaged LFP waveforms in the contralateral S1 (cS1), ipsilateral S1 (iS1), and ipsilateral striatum (CPu). (C) Group-averaged ΣLFP time courses in the cS1, iS1, and CPu ($n=5$). Stimuli fixed at 10 mA and 12 Hz. Error bars (labeled in deeper colors) are s.e.m. values.

was close to iS1 ROI. No hyper-perfusion was found on day 28 ($P>0.05$).

Striatal and Cortical Functional Cerebral Blood Volume Changes after Stroke

In the 20-minute MCAO group ($n=5$), stimulation at 12 Hz, 10 mA, and 3 milliseconds pulse width was applied to both forepaws. At the control time point, bilateral CBV increases were shown in the S1 and bilateral CBV decreases were shown in the CPu (Figure 5A and Figure 6A). After stroke, the CBV fMRI data acquired immediately after reperfusion (day 0) showed intact functional responses in the area contralateral to the stroke side (cS1 and cCPu), while the stimulus-evoked CBV increases in the iS1 reduced significantly compared with the contralateral side and the iS1 response acquired at the prestroke control time point ($P<0.05$). The CBV decreases in the iCPu was completely eliminated on day 0 ($P<0.05$). Compared with day 0, the iS1 response recovered significantly on day 7 ($P<0.05$). No significant difference was found between cS1 and iS1 responses on day 7 ($P>0.05$), but only on day 28 ($P<0.05$). Interestingly, iCPu was hyper-reactive and showed stronger CBV decreases compared with the contralateral side, prestroke control data, and day 0 ($P<0.05$). On day 28, the iCPu response was weaker than that on day 7 ($P<0.05$) and showed no significant difference compared with control ($P>0.05$).

In the 45-minute MCAO group ($n=5$), identical stimulation was applied (Figure 5B and Figure 6B). Similar to the 20-minute MCAO group, the CBV fMRI data, acquired immediately after reperfusion

(day 0), showed no apparent functional response in both iS1 and iCPu compared with the intact side ($P<0.05$). However, the iS1 and iCPu responses did not recover up to 28 days. Significant difference was found between the two hemispheres at all time points ($P>0.05$). Although, there is a tendency that the responses in the iS1 and cS1 gradually increased over time, no significant difference was detected. Compared with the 20-minute MCAO group, the CBV decreases in iCPu was significantly less on day 7 and day 28 ($P<0.05$).

Striatal Local Field Potential Changes after Stroke

To verify whether striatal neuronal function is recovered, LFPs were recorded at the iCPu in a separate group of animals, underwent 20-minute MCAO ($n=4$). Identical stimulation parameters were used. ΣLFP at the control time point showed no statistical difference compared with the normal control data in the first study (see Figure 3A, $P>0.05$). ΣLFP decreased significantly after stroke (day 0, $P<0.05$). On day 7, ΣLFP showed a trend of recovery, but did not reach a significant level ($P=0.07$). On day 28, significant functional recovery was observed compared with day 0 ($P<0.05$) and no significant difference was detected compared with prestroke control time point (Figure 6C).

DISCUSSION

This study established a longitudinal fMRI protocol to study the rat striatum under isoflurane anesthesia. In normal rats, neurovascular

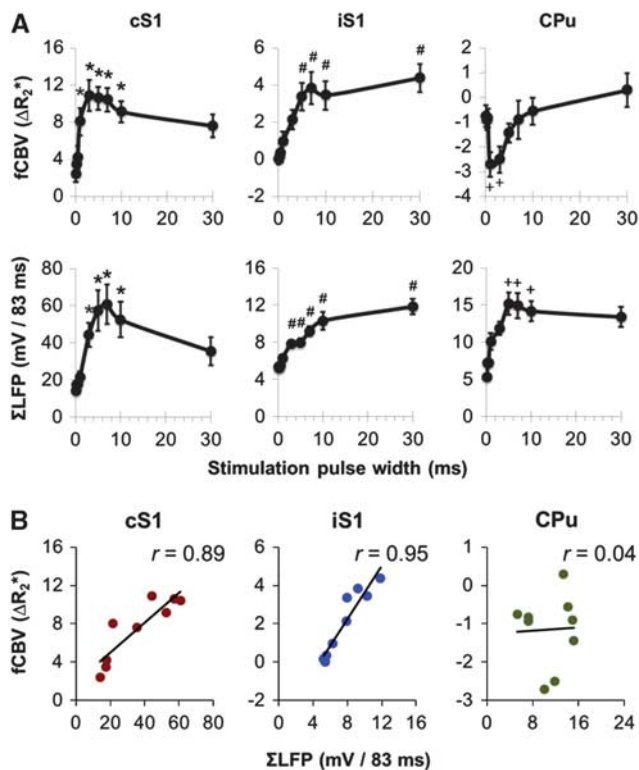


Figure 3. Comparison of cerebral blood volume functional magnetic resonance imaging (CBV fMRI) and Σ LFP. (A) Pulse-width-dependent CBV fMRI and Σ LFP responses. $P < 0.05$ indicates statistical significance. *different from 0.1 to 0.5 milliseconds; #different from 0.1 to 1 milliseconds; + different from 0.1 to 0.5 and 30 milliseconds. (B) Correlation analysis revealed significant neurovascular coupling in the contralateral S1 (cS1) and ipsilateral S1 (iS1; both $P < 0.05$), but not in the ipsilateral striatum (CPu; $P > 0.05$). LFP, local field potential.

function in the cortex and the striatum was evaluated by correlating CBV fMRI response with LFP results. This fMRI procedure was further applied to study striatal functional recovery in an ischemic stroke model with different occlusion time. The major findings were: (i) Cortical and striatal fMRI responses can be reliably and longitudinally obtained in rats under isoflurane anesthesia; (ii) the positive CBV responses in the S1 peaked ~ 3 to 10 milliseconds pulse width when 12 Hz and 10 mA stimulation was applied, while the negative CBV responses in the CPu peaked ~ 1 to 3 milliseconds pulse width; (iii) strong CBV–LFP correlations as a function of pulse width were observed in the cS1, iS1, but not in the CPu; (iv) this fMRI procedure provided additional information than ADC and CBF to characterize potential tissue abnormalities, offering a means to evaluate striatal dysfunction in stroke rats; (v) both fMRI and LFP responses were diminished immediately after stroke and recovered on day 28; and (vi) fMRI data showed that the 20-minute MCAO group had better recovery than 45-minute MCAO group.

Neurovascular Responses in the Striatum

The striatum involves in various aspects of brain signaling. We previously found that unilateral noxious electrical stimuli at the rat forepaw reliably evoked bilateral negative CBV responses in the striatum under alpha-chloralose anesthesia.⁶ These responses correlated with increased neuronal spike activity and increased Fos-immunoreactive cells. Consistent with the negative CBV responses, negative BOLD^{2–4,7,8} and CBF fMRI changes² were

also found in the striatum during stimulation. These data implied a unique control of the striatal circulation and the mechanism of action is potentially via direct control of local vasculature rather than conventional neurovascular coupling because striatal spike activity⁶ or metabolism² were not reduced during stimulation. It has been shown that pain signaling may trigger this local vascular control. Morphine, a μ -opioid receptor agonist and a commonly used pain reliever, enhanced the stimulus-evoked negative striatal CBV responses and naloxone, a μ -opioid receptor antagonist, eliminated the evoked striatal fMRI responses.⁵ Antagonism of dopamine D₂-like receptor markedly enhanced pain-related behavior.²⁴ This was not surprising because opioid and dopamine D₂ systems orchestrate closely. Acute upregulation of D₂-like, but not D₁-like receptors was found to occur after administration of μ -opioid receptor agonist.²⁵ Positron-emission tomography studies using dopamine D₂-like radioligands also support this notion by showing that administration of a μ -opioid receptor agonist significantly increased D₂-like receptor binding in both the cortical and the striatal areas.²⁶

Although, little is known about this unique hemodynamic regulation in the striatum, activation of D₂-like receptors has been shown to induce striatal CBV decreases.²⁷ Injection of D₂-like receptor antagonist also reduced the striatal negative fMRI responses evoked by noxious forepaw stimuli.^{5,6,10} These results, in line with an earlier report,²⁸ implied that dopamine and its receptors can regulate regional microcirculation. We recently showed that the striatal CBV decreases in this stimulation model is associated with the integrity of the dopaminergic innervation.¹⁰ The dopaminergic afferents in the striatum were selectively lesioned by injecting the 6-hydroxydopamine to the substantia nigra pars compacta, which sends long projecting dopaminergic efferent fibers to the striatum. Our data showed that the stimulus-evoked striatal CBV reduction was almost eliminated on the lesion side, where the cCPu still showed intact fMRI response. The remaining fMRI response showed very high spatial correlation with the residual dopaminergic fibers as revealed by tyrosine hydroxylase immunostaining.

In this study, we further investigated the striatal fMRI responses with modulation of nine different pulse widths and showed that the striatal CBV reduction is also evident under isoflurane anesthesia at certain pulse widths. Frequency, amplitude, and pulse width are critical parameters for electrical stimuli. It has been shown that 12-Hz forepaw stimuli evoked robust BOLD and CBF changes in the S1 under isoflurane anesthesia compared with other frequencies (3, 6, and 20 Hz).¹⁴ The striatal CBV reduction monotonically enhanced with the stimulus amplitude under different anesthetics/sedative.^{2,4,6} However, the stimulus pulse width that determines the amount of charge delivered has not been previously evaluated. It has been shown that at different stimulus pulse widths, the nerve fibers recruited by the stimuli were also different.²⁹ A typical example is that peripheral sensory fibers are more effectively evoked with longer pulse width than motor fibers.³⁰

Our fMRI data showed that the cS1 CBV response exhibited two phases during pulse-width modulation: in phase 1, the responses increased with pulse width and peaked ~ 3 milliseconds. In phase 2, the responses decreased until 30 milliseconds (the longest pulse width measured in this study). The increasing response in phase 1 was because of a larger amount of charges being delivered to the forepaw and potentially recruited more sensory units sending action potentials to the S1 region. In contrast, the decreasing response in phase II may be because of effective refractory period since long pulse width has been shown to extend the recovery time course from neural refractoriness.³¹ This was also evident in our LFP measurements, showing reduced Σ LFP (less synaptic currents being generated) in the cS1 at 30 milliseconds pulse width. Similarly, the fMRI response in the iS1 increased with pulse width, but it did not decline up to 30

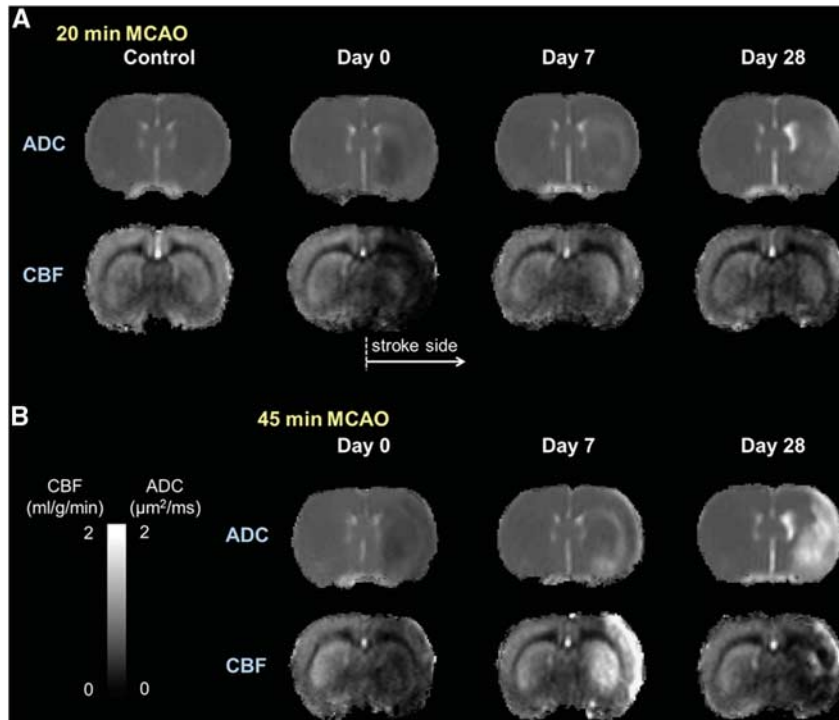


Figure 4. Group-averaged cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) maps at different time points. Middle cerebral artery occlusion (MCAO) was performed to induce stroke on the right hemisphere; **(A)** 20-minute MCAO group ($n = 5$) and **(B)** 45-minute MCAO group ($n = 5$).

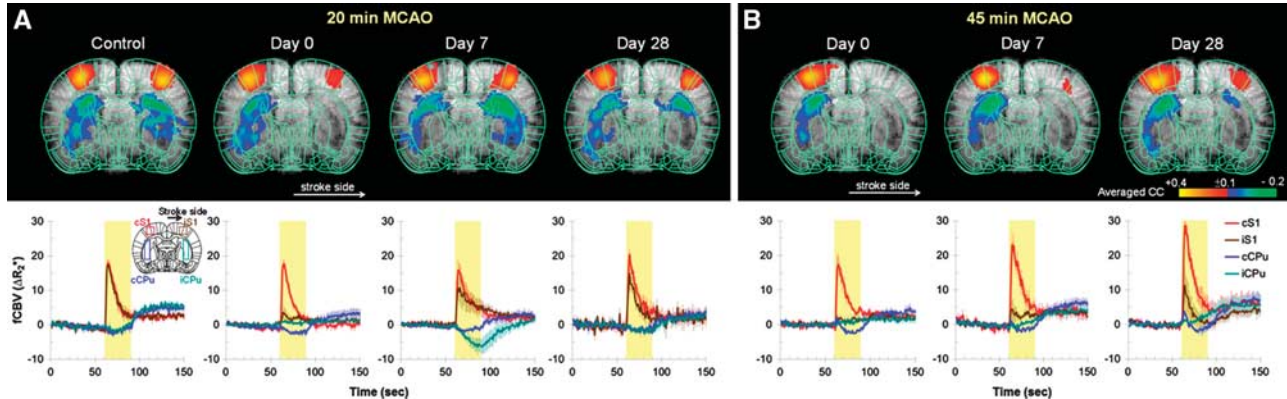


Figure 5. Cerebral blood volume functional magnetic resonance imaging (CBV fMRI) responses of the **(A)** 20-minute middle cerebral artery occlusion (MCAO) group ($n = 5$) and **(B)** 45-minute MCAO group ($n = 5$). CBV fMRI images are expressed as coregistered group-averaged maps of the correlation coefficient. Stimulation was applied simultaneously at two forepaws with 10 mA, 12 Hz, and 3 milliseconds pulse width. Control images were acquired 3 to 5 days before stroke. Images acquired immediately after MCAO and reperfusion was designated as day 0. Averaged CBV fMRI time courses at corresponding time points are shown below. Regions of interest are shown in inset. The yellow-shaded regions indicate stimulation period. The unit of y-axis is in per second. Error bars are s.e.m. values. cCPu, contralateral CPU; cS1, contralateral S1; iCPu, ipsilateral striatum; iS1, ipsilateral S1.

milliseconds pulse width. Cerebral blood volume response at the iS1 can be evoked by the pain pathway that projects bilaterally, subsequently evoked by the cS1 via callosal projections, or because of nonspecific cardiovascular effect.^{32–34} It has been shown that transient blood pressure changes could affect fMRI signals and the stimulus-evoked activation could overlap with MR signal changes because of MABP increases.³³ Despite our electrophysiological data showed changes in LFP, and the fMRI responses are highly localized in the target area of interest.

We cannot rule out the potential contribution of cardiovascular effects on all of the fMRI data presented herein. The Σ LFP peak response in the iS1 was several times lower than that the contralateral side and the responses were far from fully saturated, so the group neural refractoriness was not obvious. Both the fMRI pulse-width modulation curves in the cS1 and iS1 were highly correlated with the Σ LFP. These data from the cortex also served as within-subject controls for our measurement in the striatum.

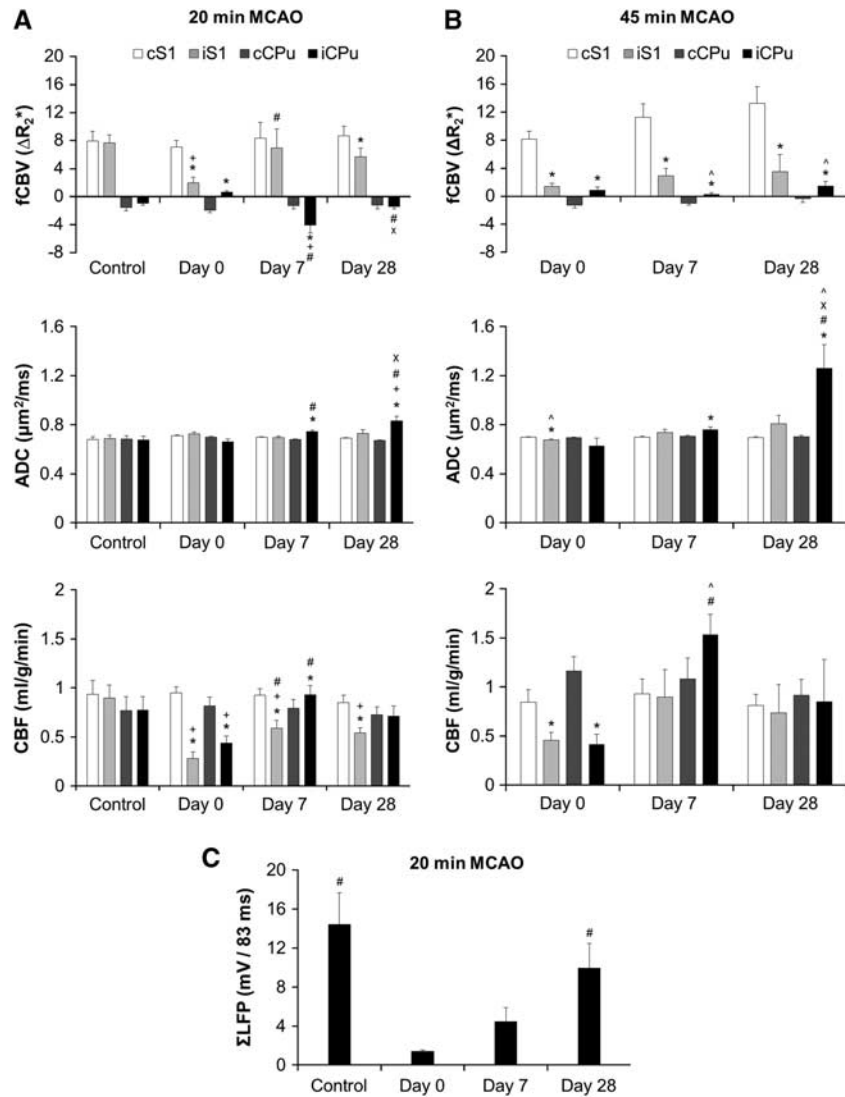


Figure 6. CBV functional magnetic resonance imaging response, ADC, and CBF in (A) 20-minute MCAO ($n = 5$) and (B) 45-minute MCAO group ($n = 5$). The regions of interest are the same as shown in Figure 4B. (C) Σ LFP in 20-minute MCAO ($n = 4$), where the recording electrode was implanted into the iCPu. $P < 0.05$ indicates statistical significance. *different from contralateral side; + different from control; #different from day 0; ^different from day 7; Xdifferent from the matched time point in the 20-minute MCAO group. Error bars are s.e.m. values. ADC, apparent diffusion coefficient; CBF, cerebral blood flow; CBV, cerebral blood volume; cCPu, contralateral CPu; cS1, contralateral S1; fCBV, functional CBV; iCPu, ipsilateral striatum; iS1, ipsilateral S1; LFP, local field potential; MCAO, middle cerebral artery occlusion.

Interestingly, the striatal CBV response showed a band-pass shape in pulse-width modulation and was only evident at 1 to 3 milliseconds pulse widths. Little or no CBV reduction was observed with extremely short (0.1 milliseconds) or long pulses (30 milliseconds). The mechanism leading reduced negative CBV response at long pulse width is not elusive and warrants further investigation. fMRI pulse-width modulation curve in the striatum did not correlate with the corresponding LFP measurement. During the modulation of pulse width, Σ LFP responses were positive, while the fMRI responses were mostly negative. The shape of the fMRI pulse-width modulation curve may be attributed to the nonspecific cardiovascular effect, whereby longer stimulus pulse width may evoke subsequent positive hemodynamic responses and eliminate striatal negative CBV changes. It is also plausible that the D_2 signaling cascade in the basal ganglia circuit simply preferred 1 to 3 milliseconds pulse width under our experimental condition. We speculate that the negative fMRI response in the striatum is resulted from the process of the local

vasculature that overrides the existing neurovascular coupling because fMRI responses were neither positively, nor negatively correlated with the Σ LFP. Given the striatum has dense and uniform distribution of the dopamine D_2 receptors,³⁵ this unique signal pattern therefore has the opportunity to serve as an imaging marker to evaluate striatal function and functional recovery *in vivo*. On a separate note, although CBV responses were consistently observed in the S1 and CPu under our stimulation protocol, CBV response in the thalamus was not detected. The S1 and striatum are both important regions in the pain network,^{22,36,37} in which the S1 is in the lateral pain system discriminating the location and intensity of the stimuli,³⁸ whereas the striatum receives strong projections from the medial pain system.³⁹ We did not detect thalamic activation, consistent with a prior study in rats.³⁶ The absence of thalamic activity could be because of the effect of isoflurane on suppressing thalamic activity⁴⁰ and/or regional dependence of neurovascular coupling.⁴¹

Striatal Functional Recovery after Stroke

Our data showed that forepaw electrical stimuli at 12 Hz, 10 mA, and 3 milliseconds pulse width generated robust striatal CBV reduction under isoflurane anesthesia. Three milliseconds pulse width also generated robust positive fMRI responses in the cortex, so the stimulus parameters do not need to be compromised for assessing the function of cortical or striatal region. This protocol is noninvasive and can be used longitudinally.

To show the feasibility of using this stimulus protocol for assessing striatal functional recovery in a longitudinal manner, two groups of rats underwent MCAO (20 and 45 minutes, respectively) followed by reperfusion were used. The stimulation protocol is essentially identical except both forepaws were stimulated simultaneously in series to evoke S1 activation at both hemispheres. Apparent diffusion coefficient and CBF showed similar trend as our previous reports.⁴² In the 45-minute MCAO group, postischemic hyper-perfusion was found on day 7 and striatal ADC was significantly higher on day 28 compared with the 45-minute MCAO group. As expected, there was a tendency that the 20-minute MCAO group (Figure 4) showed better functional activation and more robust fMRI time course in the iS1 at chronic time points (day 7 and day 28) compared with the 45-minute MCAO group (Figure 5), indicating a better functional recovery. Similarly, the striatal response also showed better recovery in the 20-minute MCAO group. The ipsilateral negative CBV responses in the striatum were quantitatively stronger on day 7 and day 28 in the 20-minute MCAO group. The between-group difference in the iS1 did not reach statistical significance. This may be because of the nature of the MCAO model that the boundary of the ischemic-reperfusion insult is usually at the forepaw area of S1 in our experimental condition.⁴² Functional recovery/reorganization of the somatosensory cortex after the stroke has been studied extensively^{42–46} and it was expected that shorter MCAO resulted in significantly better functional recovery.

In the 20-minute MCAO group, the ipsilateral striatal negative fMRI responses were diminished on day 0, hyper-reactive on day 7, and recovered on day 28. The abnormality of striatal fMRI responses after stroke could represent impairments of the vascular reactivity or neural dysfunction. Our data suggested that this abnormality could have a neural origin as the LFP response was also diminished on day 0 and recovered on day 28. However, a more thorough investigation on this topic is required to identify the cellular underpinnings of the negative fMRI signals in disease states. In the 45-minute MCAO group, the striatal negative fMRI signals did not recover on day 28, showing that these signals can be manipulated by disease severity. We suspect that the enhanced-negative fMRI response on day 7 in the 20-minute MCAO group is similar to our previous findings in normal subjects, because of the dopamine receptor function. Striatal D₂-like receptor has been reported to upregulate in Parkinson's disease so as to compensate the reduced dopamine release in the striatum.⁴⁷ In contrast, the D₂ receptor has been found to downregulate when the animals were chronically exposed to quinpirole (a D₂-like agonist) for 6 days.⁴⁸ In a rat model of MCAO, newly generated striatal neurons expressing dopamine D₂-like receptors was found to develop new efferent projections to the substantia nigra in the adult rat brain after stroke,⁴⁹ indicating a potential involvement of the D₂-expressing neurons in restoring striatal function. A potential limitation of the present study is that bilateral forepaws were stimulated simultaneously to minimize the scan time for stroke rats. This, however, precluded the opportunity to identify whether the striatal hypersensitivity in the 20-minute MCAO group on day 7 was in result of unilateral or interhemispheric reorganization.

CONCLUSIONS

This study established an fMRI procedure to longitudinally study striatal neurovascular function under isoflurane anesthesia and

showed a novel fMRI application to investigate the striatal functional integrity after 20- or 45-minute MCAO. One advantage of this procedure is that no receptor-specific pharmaceutical ligand is involved and the response is evoked via peripheral stimuli reflecting endogenous brain signaling. High CBV-LFP correlation was found in the somatosensory cortex, but not in the striatum during stimulus pulse-width modulation. Our data unambiguously showed that negative CBV response could appear with positive LFP changes. We suspect that the negative fMRI response in the striatum originates from direct modulation of local vascular activity and overrides the activation-induced hyperemia. Our approach offers a means to evaluate striatal dysfunction and functional recovery in animal models. Future studies will combine optogenetic and pharmacogenetic techniques to dissect the contribution of different striatal neurons to the negative fMRI signals.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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