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Zhiguo Li
Qin Huang
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Zhiguo Li,[a,†] Qin Huang,[b,†] Peng Liu,[c] Pengcheng Li,[b] Lianting Ma,[a,c,*] and Jinling Lu[b,*]
[a]Southern Medical University, 1838 Guangzhou Avenue North, Guangzhou 510515, China
[b]Huazhong University of Science and Technology, Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, 1037 Luo yu Road, Wuhan 430074, China
[c]Wuhan General Hospital of Guangzhou Military Command, Department of Neurosurgery, 627 Wuluo Road, Wuhan 430070, China

Abstract. Subarachnoid hemorrhage (SAH) is often accompanied by cerebral vasospasm (CVS), which is the phenomenon of narrowing of large cerebral arteries, and then can produce delayed ischemic neurological deficit (DIND) such as lateralized sensory dysfunction. CVS was regarded as a major contributor to DIND in patients with SAH. However, therapy for preventing vasospasm after SAH to improve the outcomes may not work all the time. It is important to find answers to the relationship between CVS and DIND after SAH. How local cerebral blood flow (CBF) is regulated during functional activation after SAH still remains poorly understood, whereas, the regulation of CBF may play an important role in weakening the impact of CVS on cortex function. Therefore, it is worthwhile to evaluate the functional response of CBF in the activated cortex in an SAH animal model. Most evaluation of the effect of SAH is presently carried out by neurological behavioral scales. The functional imaging of cortical activation during sensory stimulation may help to reflect the function of the somatosensory cortex more locally than the behavioral scales do. We investigated the functional response of CBF in the somatosensory cortex induced by an electrical stimulation to contralateral forepaw via laser speckle imaging in a rat SAH model. Nineteen Sprague-Dawley rats from two groups (control group, $n = 10$ and SAH group, $n = 9$) were studied. SAH was induced in rats by double injection of autologous blood into the cisterna magna after CSF aspiration. The same surgical procedure was applied in the control group without CSF aspiration or blood injection. Significant CVS was found in the SAH group. Meanwhile, we observed a delayed peak of CBF response in rats with SAH compared with those in the control group, whereas no significant difference was found in magnitude, duration, and areas under curve of relative CBF changes between the two groups. The results suggest that the regulation function of local CBF during functional activation induced by somatosensory stimulation might not be seriously impaired in the somatosensory cortex of rats with SAH. Therefore, our findings might help to understand the clinical phenomenon that DIND might not occur even when CVS was found in SAH patients. [DOI: 10.1117/1.JBO.20.9.096008]

Keywords: subarachnoid hemorrhage; laser speckle imaging; cerebral vasospasm; delayed ischemic neurological deficit; forepaw stimulation; cerebral blood flow.

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1 Introduction

Subarachnoid hemorrhage (SAH) is one of the most common hemorrhagic cerebrovascular diseases and is mainly caused by ruptured cerebral aneurysms.[1] Patients with SAH often suffer from high disability and mortality,[2] since they are not only threatened by the acute pathophysiological events, but also by delayed ischemic neurological deficit (DIND) which may affect the prognosis of SAH. Delayed cerebral vasospasm (CVS) which is the phenomenon of the narrowing of large cerebral arteries[3] such as the basilar artery (BA) often occurs after SAH. It was easy to postulate that CVS could cause the reduction of cerebral blood flow (CBF), infarction of brain, and DIND. The clinical treatments of SAH have been focusing on preventing the arterial constriction and subsequent ischemia for years. However, whether preventing vasospasm after SAH can improve clinical outcomes has been hotly debated in recent years.[4–6] An increasing number of observations suggested that vasospasm and DIND do not have a one-to-one relationship.[4] Even severe vasospasm is not always associated with DIND in clinical SAH patients.[7] Meanwhile, DIND induced by SAH may occur when CVS is not detected.[8] It is possible that the regulation of CBF in local brain parenchyma could weaken the impact of CVS on cortex function. Evaluating the changes in cortex function in an SAH animal model might help to understand the clinical phenomenon that DIND might not occur even when CVS was found in patients with SAH.

SAH patients can present focal neurological symptoms including lateralized sensory symptoms.[9–12] The somatosensory function may play an important role during the recovery of SAH patients. Many neurological behavioral scales testing sensory, motor, and reflex functions have been mainly used in

*Address all correspondence to: Lianting Ma, E-mail: mlt1937@163.com; Jinling Lu, E-mail: lujinling@mail.hust.edu.cn

†These authors contributed equally to this work.

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experimental animals and patients to detect the effects of SAH.[11,13–18] Kamp et al.[19] reported that sensory tests could display significant deficits in the murine single-blood-injection SAH model after 24 h compared with the control group. Interestingly, sensory performance was no longer significantly different between all groups after 72 h, which indicated that sensory deficits were transient. However, as far as we know, the functional imaging of somatosensory cortex in SAH animals has not been well studied. The functional imaging of cortical activation during somatosensory stimulation may reflect the function of somatosensory cortex more locally than the neurological behavioral scales do. Investigating the functional response of CBF in the somatosensory cortex induced by somatosensory stimulation after SAH may help to confirm the effect of SAH on the somatosensory cortex.

Laser speckle imaging (LSI) can image CBF with a high-spatiotemporal resolution[20] and has been widely used to investigate spatiotemporal CBF response under different physiological and pathological conditions.[21–24] In this study, LSI was used to investigate the spatiotemporal response of CBF in the somatosensory cortex induced by forepaw electrical stimulation in rats with SAH.

2 Materials and Methods

2.1 *Animals*

Nineteen Sprague-Dawley rats (body weight: 280 ± 50 g) from two groups (control group, $n = 10$ and SAH group, $n = 9$) were used in this study. The animals were kept in cages in a ventilated cabinet with standardized conditions of temperature, humidity, and light (night/day cycle; 12 h/12 h). The rats were permitted free access to food and water. All experimental procedures were reviewed and approved by the local ethical committee.

2.2 *Rat Subarachnoid Hemorrhage Model*

The SAH model was produced in rats by double injection of autologous blood into the cisterna magna after CSF aspiration. The animals were initially anesthetized with 5% chloral hydrate (0.9 ml/100 g, IP) and allowed to spontaneously breathe. With the aid of a surgical microscope (Olympus, Japan), a small (1.0 to 1.5 cm), longitudinal, midline suboccipital incision centered over the foramen magnum was made until the atlanto-occipital membrane was visualized. Cerebrospinal fluid (CSF; 0.3 ml) was withdrawn carefully from the cisterna magna followed by the injection of autologous blood to induce the first SAH. The autologous nonheparinized blood (0.3 ml) drawn from the tail artery was slowly injected into the cisterna magna (day 0) for over 2 min using a 25-gauge needle. To prevent a fistula, an absorbable sponge was used to seal the hole made by the puncture immediately after the injection of the blood. All of the procedures mentioned above were performed under sterile conditions. After the wound was sutured, the animals were positioned at a 30 deg angle with the head down in a neutral position for 30 min in order to hold the blood in the basal cisterns. After recovery from anesthesia, the rats were returned to the feeding room. Forty-eight hours after the first SAH (day 2), the second injection was performed in the same manner as the first. In the control group, the same surgical procedure was applied but without CSF aspiration or blood injection.

2.3 *Animal Preparations for Laser Speckle Imaging*

Five days after the first SAH or sham surgery, the rats were anesthetized with isoflurane inhalation (4%) in an induction chamber. Anesthesia was maintained using isoflurane (2%) in a 2:1 air to O_2 mixture with the aid of a nose cone. Body temperature was kept at $37°C \pm 0.5°C$ using a feedback-regulated heating pad (69001; RWD Life Science, China). The right femoral artery was catheterized using a PE50 tube (SCI) for blood pressure monitoring and blood sampling. The right femoral vein was also catheterized for anesthetic drug administration. The arterial blood pressure was continuously monitored (FOP-LS-2FR-10, FISO, Canada) and the mean arterial blood pressure was maintained in the range of 115 ± 5 mm Hg. Blood gas was analyzed (ABL 700, Radiometer Medical, Copenhagen) in the majority of the experimental rats. The values of arterial pH, PCO_2, PO_2 were maintained in ranges of 7.35 to 7.40 (pH), 35 to 45 mmHg (PCO_2), and 100 to 180 mmHg (PO_2), respectively. The rats were positioned in a stereotaxic frame (RWD Life Science, China). A midline incision was made to expose the surface of the skull. Over the area of the right primary somatosensory cortex, a high-speed dental drill (K1070, Foredom) was used to make a (5×8 mm^2) cranial window that was thinned to translucency. Normal saline was used for cooling during the grinding process. After surgery, isoflurane was discontinued for at least 1 h before the data measurement was performed. The anesthetic drug was switched to α-chloralose after surgery and maintained during all stimulation studies. The α-chloralose was induced intravenously first with a single bolus of 50 mg/kg and maintained with a continuous 25 mg/kg/h dose by a syringe pump (RWD402, RWD Life Science, China). If autonomic movement of the animal was detected on the body or limbs, the experiment was paused and an additional dose of α-chloralose was applied (30 to 40 mg/kg/h in 5 to 10 min) to stop the movement. Meanwhile, the blood pressure and blood gas were maintained in the controlled ranges.

2.4 *Electrical Forepaw Stimulation*

Two thin stainless steel needle electrodes were inserted under the skin between digits 2 and 3 and digits 4 and 5 in the left forepaw of the rats. Stimulation was performed using a stimulator (A-M Systems model 2100). Each stimulation trial consisted of rectangular constant current pulses (1.5 mA, 0.3 ms, 5 Hz). For each trial, it is consisted of baseline (1 s), stimulation (2 s), and poststimulus (7 s) periods. Every experiment involved 20 to 30 sequential trials and all intervals between adjacent trials were longer than 150 s.

2.5 *Imaging Procedure*

A 12-bit CCD camera (TXG04h, Baumer, Germany) mounted on a microscope (SZX12, Olympus, Japan) was used to acquire the laser speckle images (640×480 pixels) at 50 fps (exposure time $T = 5$ ms) over the thinned skull. The rat cortex was illuminated by a laser diode (660 nm, 120 mW, Thorlabs). Five-hundred consecutive frames (i.e., 10 s) of speckle images were recorded in each trial. Stimulation began 1 s after the onset of the image data recording.

2.6 *Image Data Processing*

The raw speckle images were first converted to speckle contrast images using a 7×7 pixels sliding window to calculate the

contrast value of each pixel and then averaged across 10 consequent frames to improve the signal-to-noise ratio, as described in the previous literature.[25,26] The speckle contrast images were then converted to relative cerebral blood flow (rCBF) by converting each speckle contrast value to an intensity autocorrelation decay time,[27] which was assumed to be inversely proportional to blood flow. The mean ΔrCBF within the region of interest of the control group and SAH groups were plotted. Time to peak, magnitude, and duration of ΔrCBF were studied to quantify the CBF response in each animal. Time to peak of ΔrCBF was defined as the time from the beginning of the stimulation to the maximal CBF change or the response peak. The magnitude of ΔrCBF was defined as the rate of CBF change obtained by contrasting the maximum CBF change recorded after stimulation to the average CBF value during the baseline period, which was recorded 1 s before stimulation after the onset of the image data recording. The full width at half maximum was identified as the response duration. The areas under curve (AUC) of rCBF responses during the response duration between two conditions were also studied. AUC could be regarded as a focal increase in cerebral blood volume to meet increased metabolic demands by neurons within the active regions of the brain.

2.7 *Histological Examination*

After the imaging procedure, electrical stimulation to the contralateral forepaw was stopped. The α-chloralose was maintained at least for 2 h. Then the rats in each group were killed using the fixation perfusion method.

2.7.1 *Perfusion and fixation*

A thoracotomy was performed and a cannula was placed in the left ventricle. While the abdominal aorta was clamped, the right atrium was widely opened. Perfusion was performed with 500 ml of normal saline at 37°C and was followed by 500 ml of 4% paraformaldehyde. The whole brain with BA was removed after perfusion-fixation and immersed overnight in the same fixative solution at 4°C.

2.7.2 *Hematoxylin and eosin staining (H&E staining)*

The entire length of the BA was divided into three parts: the proximal, middle, and distal thirds. Approximately 3 mm of artery tissue with the brainstem was dissected out at each midpoint of the three parts and embedded in paraffin. The paraffinized samples were sectioned at 4-μm thicknesses at the midpoint. All 4-μm thick sections were deparaffinized, hydrated, washed, and stained with H&E.

2.7.3 *Measurement of basilar artery cross-sectional area*

The cross-sectional areas of the blood vessels were evaluated using Image J software (National Institutes of Health, Bethesda, Maryland). Morphometric analysis was performed by a pathologist who was blind to the group allocation. The perimeters of the vessel lumen were measured, and equivalent r ($r = \text{radius}$) values from the perimeter measurement ($r = \text{perimeter}/2\pi$) were calculated. The areas were calculated using the equation for that of a circle (area $= \pi r^2$). This method can be used to correct for off-transverse sections and vessel deformation. For each vessel, three sections from the midpoint of the three parts (proximal, middle, and distal) were measured and averaged.

2.8 *Statistical Analysis*

Statistical analysis was conducted using SPSS 13.0. Differences between the two groups were analyzed with the ANOVA test. The differences in time to peak, magnitude, duration, AUC of ΔrCBF, and BA cross-sectional area were analyzed. Statistical differences were considered significant when $P < 0.05$. The results are expressed as mean $\pm$ SD.

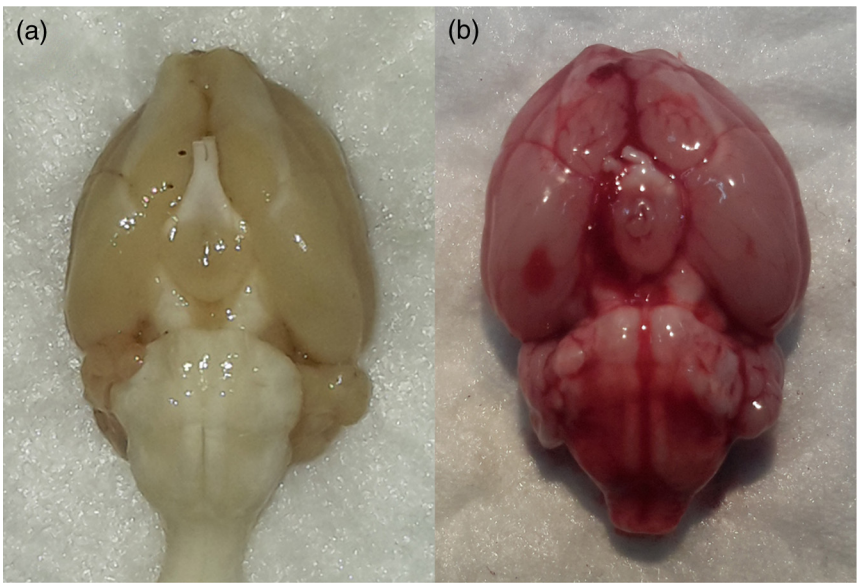

Fig. 1 Representative photographs showing: (a) a rat from the control group had no subarachnoid blood and (b) a rat from the subarachnoid hemorrhage (SAH) group presented blood in the basal subarachnoid space.

3 Results

3.1 *General Observation*

The rats in the SAH groups exhibited blood clots over the basal subarachnoid space, whereas those in the control group had no subarachnoid blood (Fig. 1).

3.2 *Basilar Artery Cross-Sectional Area*

The cross-sectional area of the BA in the SAH group ($20237.15 \pm 3877.33\ \mu m^2$) was significantly smaller than that of the control group ($61607.38 \pm 8816.50\ \mu m^2$) [$P < 0.01$, Fig. 2(c)]. Therefore, significant CVS was found in the SAH group [Fig. 2(b)].

3.3 *Functional Responses of Cerebral Blood Flow*

Figure 3 shows the spatiotemporal images of CBF response induced by forepaw electrical stimulation from two representative animals, one from the control group and the other from the SAH group. We found a local functional response of CBF in the somatosensory cortex. A sliding window of 20×20 pixels was used in the ROI. Using the curve of ΔrCBF (Fig. 4), we can quantify the ΔrCBF in a rat's somatosensory cortex through forepaw stimulation under different conditions.

In the control group, the magnitude of ΔrCBF in response to forepaw stimulation was $24.09 \pm 8.42\%$ while the magnitude of ΔrCBF increased to $30.81 \pm 9.75\%$ in the SAH group, however, there was no significant difference between the two groups [$P = 0.125$, Fig. 5(a)]. There was a significant delay in time

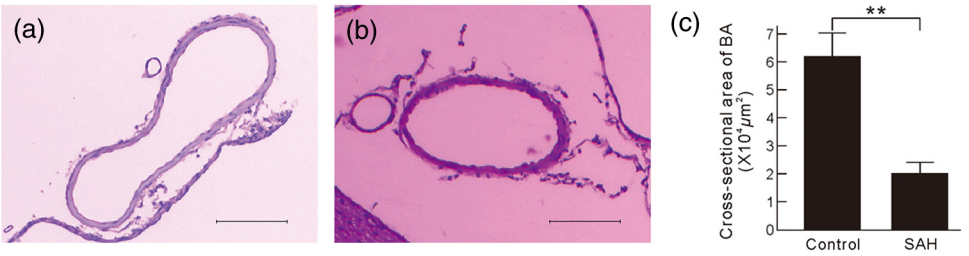

Fig. 2 Representative images of basilar artery (BA) sections from the: (a) control and (b) SAH groups showing vasospasm of large arteries after SAH (hematoxylin and eosin staining, scale bar = 100 μm). (c) Compared to the control group, the cross-sectional areas of BA were significantly decreased in the SAH group ($P < 0.01$). $^{**}P < 0.01$.

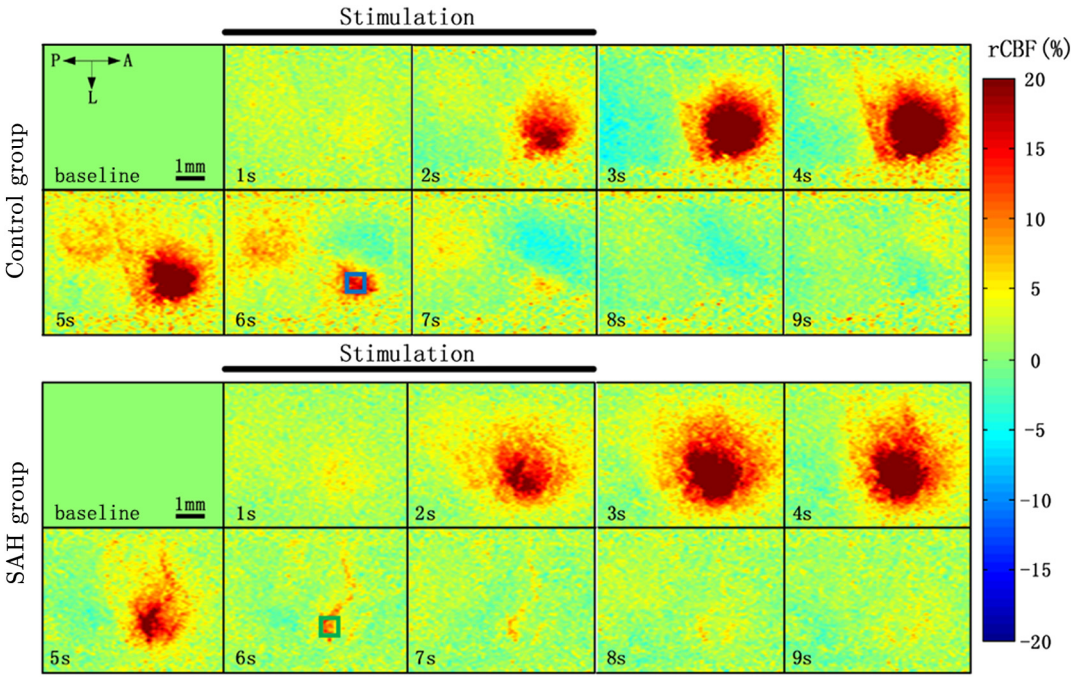

Fig. 3 Representative images from two animals, one each from the SAH and control groups, showing local changes of relative cerebral blood flow (CBF) in the somatosensory cortex. Each image represents a 1 s interval. The images indicate an increase in the CBF approximately 1 s after the stimulus onset. The time course and patterns of CBF changes are very similar between the two groups. The black bar indicates the period of electrical stimulation to contralateral forepaw. Stimulation began 1 s after the onset of the image data recording. The blue and green boxes are the selected regions of interest from which we plotted the curves of CBF change in Fig. 4. A: anterior; P: posterior; L: lateral.

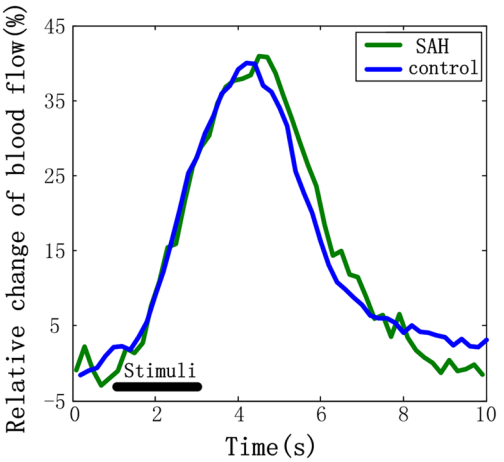

Fig. 4 Representative curves of ΔrCBF in response to somatosensory stimulation from two animals, one from the control group and the other from the SAH group. The curves during rising phase of the two rats are almost overlapped and the maximum changes of relative CBF are almost the same. However, a delay in time to peak of ΔrCBF in response to forepaw stimulation can be found in the SAH animal. Notice that these two lines start from 0.2 s, since the frame rate of blood flow images is 5 Hz.

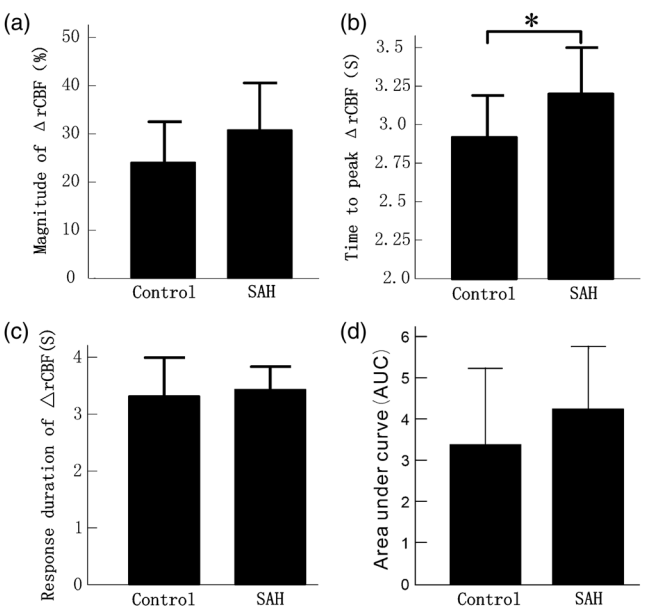

Fig. 5 (a) The magnitude of ΔrCBF for the control and SAH groups. The magnitude of ΔrCBF in response to the stimulation was not significantly influenced by SAH ($P = 0.125$). (b) The time to peak of ΔrCBF in the two groups. The time to peak of ΔrCBF increased significantly after SAH ($P = 0.047$). *$P < 0.05$. (c) The duration of ΔrCBF for the two groups. There was no significant change in the duration of ΔrCBF in the SAH group ($P = 0.666$). (d) Areas under the curve of ΔrCBF between the two conditions. AUC was not significantly influenced by SAH ($P = 0.293$).

to peak of ΔrCBF in response to forepaw stimulation in the SAH group [$P = 0.047$, Fig. 5(b)]. The time to peak of ΔrCBF was 2.92 ± 0.27 s in the control group, while it was 3.20 ± 0.30 s in the SAH group. There was no significant change in the duration of ΔrCBF in the SAH group [$P = 0.66$, Fig. 5(c)]. The duration of ΔrCBF was 3.32 ± 0.67 s in the control group and 3.43 ± 0.40 s in the SAH group. There was no significant change in AUC in the SAH group [$P = 0.293$, Fig. 5(d)]. The AUC was 3.35 ± 1.88 in the control group and 4.21 ± 1.53 in the SAH group.

4 Discussion

In order to confirm the effect of SAH on the regulation of CBF in the somatosensory cortex, we investigated the spatiotemporal response of CBF in the somatosensory cortex induced by forepaw electrical stimulation in rats with SAH by using LSI. We observed a delayed peak of CBF response in rats with SAH compared with those in the control group, whereas no significant difference was found in magnitude, duration, and AUC of the relative CBF changes between the two groups.

Localized increases in neuronal activity in healthy brains are strongly spatiotemporally correlated with localized increases in CBF and cerebral metabolic consumption of oxygen.[28] To maintain cerebral homeostasis, oxygen and glucose supplies are dynamically regulated to match nutrient delivery to the metabolic demands of active neurons.[29] The regulation of CBF is achieved by neurovascular coupling, which is a tight spatiotemporal coupling between neuronal activity and blood flow.[30] Thus, investigating the functional response of CBF in the somatosensory cortex induced by somatosensory stimulation after SAH may help to confirm the effect of SAH on the regulation of CBF in the somatosensory cortex.

A delayed peak of CBF response was found in the SAH group compared with those in the control group. It is possible that the delay of the time to peak was mainly due to a prolonged process of neural electrical signal conduction within the brain or neurovascular coupling in the cortex, because the spinal cord and peripheral nerves were normal in the healthy rats.[31] Sun et al.[32] suggested that SAH could prolong conduction along the sensory path within the brain. However, we also observed there was no significant difference in either the magnitude or duration of CBF changes between the groups, which suggested that, in the SAH group, neurovascular coupling in the somatosensory cortex could still work efficiently. Therefore, these results indicated that the prolonged process of neural electrical signal conduction within the brain may play a more important role in causing the delayed peak of CBF changes induced by sensory stimulation after SAH.

No significant difference in either the magnitude or duration of CBF changes was observed between the groups. There was no significant difference in AUC between two conditions either. Neurovascular coupling could simply represent a focal increase in CBF to meet the increased metabolic demands of neurons within the active regions of the brain. Thus, the regulation function of local CBF during functional activation induced by somatosensory stimulation might not be seriously impaired in the somatosensory cortex of rats with SAH. Meanwhile, significant CVS was found in the SAH group. Therefore, our findings might help to understand the clinical phenomenon that DIND might not occur even when CVS is found in SAH patients. These results are also in line with the previous report from Kamp et al.[19] who found that the differences in sensory or motor performance of single-blood-injection SAH animals after three days were not significant compared with both control groups. But Kamp et al.[19] also reported that the comparison of the sum of sensory and motor scores revealed significant differences. The total neurologic integrity score might indicate persistent delayed effects of SAH. To study the integration in brain functioning areas, the visual and motor functional imaging

of the cerebral cortex after SAH needs to be investigated in our future research.

Rat SAH models have been widely used to study the impact of subarachnoid blood on the brain and cerebral vasculature.[33] The blood injection model can effectively mimic the vasospasm that occurs after SAH.[34] However, it is recognized that the severity of the initial bleeding is the main determinant of the outcome of SAH.[35] Therefore, a slow blood injection rate and CSF aspiration could reduce or prevent the early severe peak in intracranial pressure. Consequently, the blood injection model may not be ideal for imitating clinical SAH, especially early brain injury, because it is difficult to cause drastic changes in the intracranial pressure and the reduction of CBF. Hence, experimental models of CVS need to be improved in order to better imitate clinical SAH, which mostly involve a direct hemorrhagic brain lesion under systolic pressure after the rupture of cerebral aneurysms.[36]

The chosen timeframe for studying the functional response of CBF 5 days after SAH was based on the reports that delayed cerebral ischemic deficits manifesting 4 to 10 days after aneurysm rupture,[37] and the delayed events of brain injury evoked by SAH are arterial vasospasms and delayed ischemic deficits that develop 3 to 7 days after the initial bleeding.[38] Some investigations have shown the presence of maximal arterial constriction on day 5 after the second blood injection on day 2 in the rat double SAH model.[39,40] Future studies should be conducted into the functional response of CBF at different times after SAH in order to understand the dynamic changes in neurological function in the regulation of local CBF during functional activation throughout the whole process of SAH.

5 Conclusions

In this study, we used the LSI technique *in vivo* to investigate the functional response of CBF in the somatosensory cortex induced by electrical stimulation to contralateral forepaw in the SAH model. We noted a delayed peak of CBF response in rats with SAH compared with those in the control group, whereas no significant difference was found in magnitude, duration, and AUC of CBF changes between the two groups. Meanwhile, significant CVS was found in the SAH group. Lastly, our findings suggest that the regulation function of local CBF during functional activation may not be seriously impaired in the somatosensory cortex of rats with SAH, which might help us to understand the clinical phenomenon that DIND might not occur even when CVS was found in patients with SAH.

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Zhiguo Li received his BS degree in clinical medical from Henan Medical University in 2000. After graduation, he worked as a neurosurgeon. He received his master's degree from Southern Medical University in 2012. Currently, he is working toward his PhD in neurosurgery at Wuhan General Hospital of Guangzhou Military Command. His research interests include basic and clinical studies on cerebrovascular diseases.

Qin Huang received his BS degree in physics from Jianghan University in 2011. Currently, he is working toward his PhD in optical engineering at Huazhong University of Science and Technology. His research interests include functional imaging of neural activity and laser speckle imaging of blood flow. He is a student member of the Optical Society of America.

Peng Liu is working as a neurosurgeon in Wuhan General Hospital of Guangzhou Military Command. He received his master's and PhD degrees from Southern Medical University in 2004 and 2011, respectively. He completed his postdoctoral research in the State University of New York at Stony Brook in 2012. His research interests include basic and clinical research of cerebral aneurysms, carotid artery stenosis, and other cerebrovascular diseases, etc.

Pengcheng Li is a professor at Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China. He received his MS and PhD degrees from Huazhong University of Science and Technology, China, in 2000 and 2003, respectively. His research interests include laser speckle imaging of blood flow in biological tissue and *in vivo* optical imaging of the cerebral architecture and dynamics in normal and disease brain.

Lianting Ma is a professor at both Southern Medical University and Tongji Medical College, Huazhong University of Science and Technology. He is the leading professor in the Department of Neurosurgery in Wuhan General Hospital of Guangzhou Military Command, Wuhan, China. He received his BS degree from Henan Medical University, China, in 1962. His research interests include basic and clinical research of cerebrovascular diseases.

Jinling Lu is a lecturer at Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China. She received her MS and PhD degrees from Huazhong University of Science and Technology, China, in 2003 and 2007, respectively. Her research focuses on optical imaging of cortical functional connectivity.