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TRACING COLLATERAL CIRCULATION AFTER ISCHEMIA IN RAT CORTEX BY LASER SPECKLE IMAGING

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The collateral circulation is crucial for the pathophysiology and outcome of acute cortical ischemia. Current understanding of collateral circulation still remains sparse, largely due to prior limitations of spatial or/and temporal resolution in methods to evaluate these diminutive redistributive routes of cerebral blood flow (CBF) especially in leptomeningeal anastomoses that connected cortical arteries. In the study, based on a mini-stroke model, laser speckle imaging with high spatiotemporal resolution was used to assess the dynamic evolution of the collateral circulation around a mini-ischemia in the rat cortex. We found that the blood flow and diameter in the intra-arterial anastomoses were enhanced immediately after the ligation of one branch of middle cerebral artery and recovered to baseline level as arterial recirculation was performed. Whereas the communicative flow-through of the posterior cerebral artery and the middle cerebral artery anastomoses was not significant enough to be determined. This is the evidence that intra-arterial anastomoses were the primary routes to restore blood flow into the ischemic territory during the acute phase of ischemia, and laser speckle imaging method was proven as a powerful tool to be potential for subserving further investigation of the collateral circulation.

Keywords: Cerebral blood flow; anastomoses; mini-stroke model.

1. Introduction

The cerebral collateral circulation is recruited as a subsidiary vascular network to stabilize the local cerebral blood flow (CBF) when the principal supplying arteries are constricted or occluded.[1] The supplying arteries in the brain are interconnected by two major collateral systems. One is the primary collaterals, which include the communicating arterial segments of the circle of Willis at the base of the brain, and is responsible for the redistribution of blood flow under conditions of any occlusion of the carotid or vertebral arteries. The other is the secondary collateral system that mainly derives from the leptomeningeal anastomoses which interconnect the

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distal branches of the cortical vessels. The capacity of these anastomoses is a critical determination of the volume and severity of focal ischemic injury.[2,3] Although the collateral circulation involves a crucial role in the pathophysiology of ischemia in the brain, the knowledge of these collaterals is not well known.[4]

Characterizing the collateral blood flow during ischemia will provide useful information to guide therapeutic interventions, and to promote prognostication and risk stratification of stroke in clinical practice.[1,3] Conventional angiography is regarded as the gold standard for collaterals visualization, however, objective diagnostic criteria for collaterals in clinic are rarely formulated.[1] Other angiographic or perfusion evaluating techniques, such as transcranial Doppler ultrasonography (TCD), computerized tomography angiography (CTA), magnetic resonance angiography (MRA), positron emission tomography (PET), laser-Doppler flowmetry (LDF), have limited spatial resolution for characterization of leptomeningeal and other secondary collateral pathways.[1] Meanwhile, these current diagnostic modalities also lack the timing of the dynamic evolution of the collaterals after ischemia in brain because of their relative low temporal resolution.[5] Therefore, the limited information regarding collaterals can be accrued by further studies employing higher spatiotemporal resolution techniques rather than current methods.

Optical imaging techniques, such as laser speckle imaging (LSI),[6] employing high spatial and temporal resolution (up to several micron and several microsecond), were widely used to continuously trace hemodynamic response in the brain under the physiological and pathological conditions.[7–9] Especially, laser speckle imaging is a full-field technique removing the need of scanning, as well as in real-time mode to acquire dynamic cerebral blood flow images. In this study, laser speckle imaging with high spatiotemporal resolution was introduced to assess the dynamic evolution of the collateral circulation around a mini-ischemia in the rat cortex.

2. Materials and Methods

2.1. *Animal preparation*

Eight male Sprague-Dawley rats weighing between 200 g and 280 g were used in the experiments. All experimental procedures were conducted in conformity with the Institutional Animal Care and Use Committee of Hubei Province. The rats were intraperitoneally anesthetized with a mixture of α-chloralose (50 mg/kg) and urethane (600 mg/kg) and maintained in a state unresponsive to toe-pinching. The body temperature, arterial blood pressure, pH, pCO2, and pO2 were monitored and maintained within normal limits throughout the experiments. Each rat was firmly mounted in a stereotaxic apparatus. The skull bone was removed by a craniotomy over the parietal cortex with a high-speed dental drill (Fine Science Tools, USA) under cooled saline, keeping the dura intact. Cranial windows of 6 mm $\times$ 8 mm in size were formed and then bathed with artificial cerebrospinal fluid (aCSF) at 37°C.

2.2. *Mini-stroke model in rat cortex*

The procedure of mini-stroke model was described in detail in our previous work. Firstly, short pieces of 11-0 suture thread were passed through the dura and under one selected branches of the middle cerebral artery (MCA) and left to complete the ligation.[10] Then, a piece of nylon thread (0.2 mm in diameter) was positioned on the dura over the selected branch of MCA and between the open ligature ends. Finally, the occlusion of MCA's branch was performed by tying a ligature loop to include the nylon thread, the dura and the branch of MCA.[11] After 1 hour of ischemia, the suture over the nylon thread was cut by a surgical scalpel. Then, the broken loop and the nylon thread were picked up as carefully as possible so as to resume the recirculation of the compromised arteriole.

2.3. *Laser speckle imaging*

As an interference pattern, laser speckle is produced by reflected or scattered light when a coherent laser beam illuminates a rough surface. The laser speckle pattern was further formed in the imaging equipment (such as charge-coupled device, CCD) by integrating the intensity of light fluctuation at each pixel over a finite time. The motion of scattered particles in the imaged area could blur these speckle patterns by varying the intensity of light in spatial and temporal dimensions.[6,8,9] Laser speckle imaging techniques using spatial or temporal statistics of time-integrated speckle have been developed to indentify speckle blurring that contains the moving information of the inherent particles. This blurring is represented as the local spatial or temporal speckle contrast. The spatial blurring of the speckles was quantified in the laser speckle contrast analysis (LASCA) method[6,8] by local spatial speckle contrast (K_s), which is defined as the ratio of the standard deviation (σ_s) to the mean intensity ($\langle I_s \rangle$) in a small region of the image: $K_s = \frac{\sigma_s}{\langle I_s \rangle}$. In our previous works, laser speckle temporal contrast analysis (LSTCA) was developed to construct speckle temporal contrast image by calculating the speckle temporal contrast of each image pixel successively over time.[12,13] The value of the speckle temporal contrast K_t at pixel (x, y) was calculated as

$$K_t(x,y) = \sigma_{x,y}/\langle I_{x,y} \rangle = \sqrt{\frac{1}{N-1}\left\{\sum_{n=1}^{N}\left[I_{x,y}(n) - \langle I_{x,y} \rangle\right]^2\right\}} \Big/ \langle I_{x,y} \rangle \quad (1)$$

where $I_{x,y}(n)$ is the CCD count at pixel (x, y) in the nth laser speckle image, N is the total number of images, and $\langle I_{x,y} \rangle$ is the mean value of CCD counts at pixel (x, y) over the N images. The image of inverse correlation time values (ICT, $1/\tau$, arbitrary units) calculated from the speckle contrast images[6,8] is assumed to be proportional to the regional CBF, and then used as an indicator of the CBF parameter to visualize two-dimensional evolution with a high spatial and temporal resolution. Previous studies demonstrated that the LSTCA

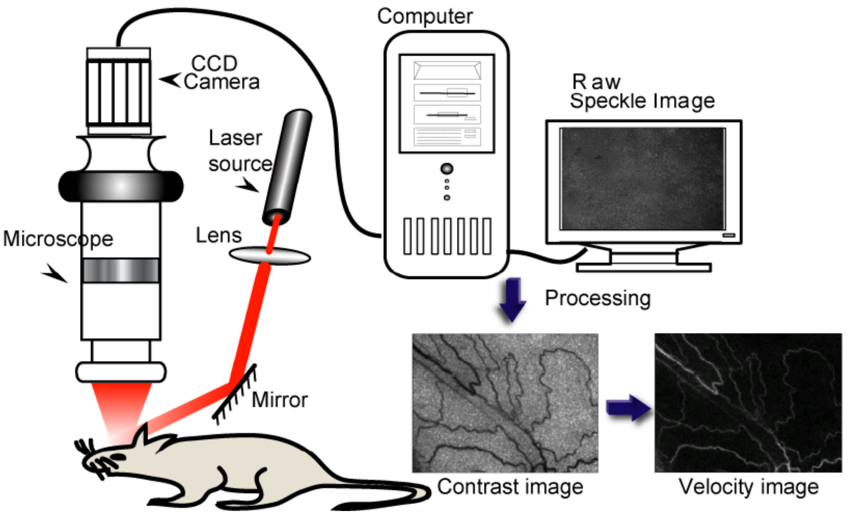


Fig. 1. The schematic setup of the laser speckle imaging system. A He–Ne laser ($\lambda = 632.8$ nm, 3 mW) beam is expanded to illuminate the exposed cortex. With a CCD camera, the raw speckle images was acquired and then processed into contrast image and relative blood flow maps by computer.

method can provide even 5 times higher spatial resolution[12] than the conventional LASCA method and can suppress the influence of the speckle contrast from a stationary structure.[13] Therefore, the LSTCA method was adopted in this study.

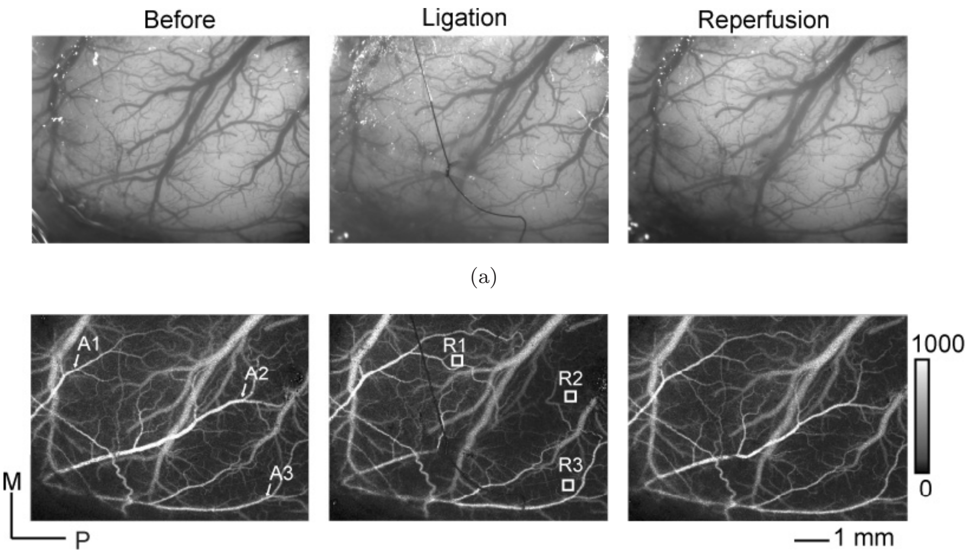
The schematic diagram of the LSI setup is shown in Fig. 1. A He-Ne laser beam ($\lambda = 632.8$ nm, Melles Griot, USA) was expanded by lens, and guided to illuminate the observed cortex in a diffuse and even manner. Raw speckle images were recorded by a 12-bit CCD camera (480 $\times$ 640 pixel, Pixelfly, PCO, Germany) through a stereoscopic microscope (SZ6045TRCTV, Olympus, Japan). The exposure time of the camera is set to 20 milliseconds, and the acquiring rate is 40 fps. The image acquisition commenced 5 minutes before the procedure of vascular occlusion and continued within 10 minutes after the ischemia or reperfusion for 30 frames per time point at an interval of 1 minute. The period of ischemia and the observed window for reperfusion both last about 60 minutes. The acquiring interval for 10 minutes to 60 minutes after ischemia or reperfusion is about 5 minutes.

2.4. *Data analysis*

For a comparison of changes in ICT value (assumed to be proportional to the regional CBF) before and up to 1 hour after occlusion, and up to 1 hour after reperfusion, several small size ($\sim$10 $\times$ 10 pixels) regions-of-interest (ROIs) were drawn within the different branches of MCA in the rat cortex or at the parenchyma regions around those 3 branches.

3. Result

The regional blood flow over the parietal cortex throughout the procedure of temporary occlusion of the MCA was studied with the LSI method. White light images of the observed cortex during baseline, immediately after ligation and reperfusion are shown in Fig. 2(a). The corresponding processed speckle images (Fig. 2(b)) under each white light image indicate the distribution of relative cerebral blood flow. The entire imaged window presents an approximately $6\,\text{mm} \times 8\,\text{mm}$ field-of-view. The darker areas in these images indicate slower blood flow, while the brighter areas show higher blood perfusion level. From these images, 3 main branches of MCA could be identified according to the morphological characteristic and the inherent higher blood flow rate than the vein. As seen in the middle image of Fig. 2(a), the ligation was performed by including a piece of nylon thread and the middle branch of MCA. The occluded branch of MCA was seen clearly in the white light images throughout the ligation procedure. So, it is hard to get any indications of the perfusion disruption of MCA's branch from the white light images. Comparing with the white light images, the downstream part of the MCA's middle branch from the ligation site almost disappeared in the ICT image (Fig. 2(b), middle image) immediately after the ligation was performed, implying little or no blood flow through it. The recovering of blood flow in the occluded vessel was then



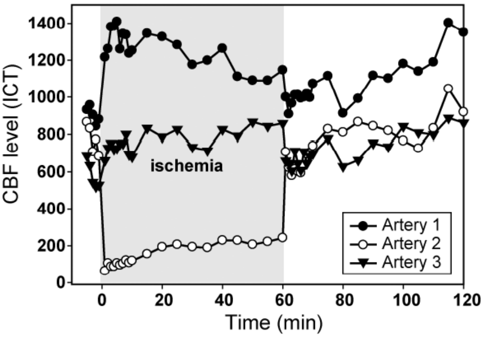
(a)

(b)

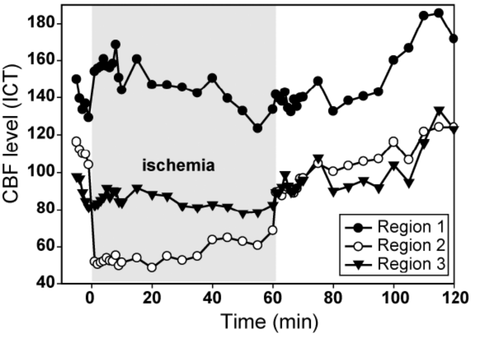
Fig. 2. White light images (a, upper row) and the corresponding relative CBF (ICT) images (b, bottom row) acquired before, after ligation, and after recirculation of the middle branches of MCA in the imaged cortex. The 3 branches of MCA were indicated by white arrows and labeled as A1, A2, and A3 respectively. Three regions around the three branches of MCA were selected for calculating the time course of changes in blood flow. M, medial; P, posterior; Bar, 1 mm.

indicated by the reappearance in the ICT image as the ligature was cut and the recirculation was performed.

To investigate the temporal variation of relative blood flow within the vessels and the parenchyma of the cortex, a set of regions-of-interest (ROIs) were drawn on the 3 branches of MCA (denoted as A1, A2, A3 in Fig. 2(b)), and on 3 around no-vessel cortical regions (denoted as R1, R2, R3 in Fig. 2(b)). The relative CBF value (ICT units) within the distal part of the compromised branch of MCA (A2) and the around region (R2) dropped drastically after ligation was performed including this branch (from 712, 110 to 60, 51 ICT units, respectively, Figs. 3(a), 3(b)). Subsequently, a progressive inverse of the CBF decrease during the ischemic period was observed in the same vessel and region. Meanwhile, the CBF proportional values in the adjacent branches (A1, A3) and around the cortical region presented an immediate increase following the ligation procedure. After reperfusion, the CBF value in all ROIs concurrently returned to baseline levels (Fig. 3).



(a)



(b)

Fig. 3. The time course of relative cerebral blood level in the different regions-of-interest (ROIs) that selected in the imaged cortex and denoted in Fig. 2(a) Changes in CBF level in the 3 branches of MCA. (b) Changes in CBF level in the 3 regions of interest. The shade of the time period corresponded to the duration of ischemia.

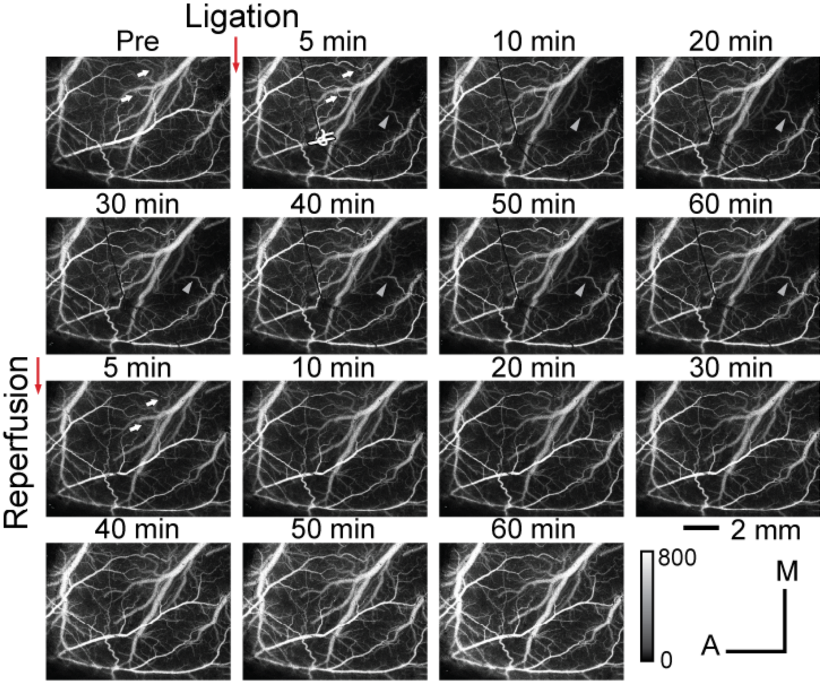


Fig. 4. The spatiotemporal evolution of the distribution of CBF in the imaged cortex during the time range from before ligation, to 60 minutes after ligation, and to 60 minutes after reperfusion. The white arrows indicate the intra-arterial anastomoses within MCA, and the white triangle denotes the progressive restoring of CBF in the occluded branch during the ischemic period. The time points were labeled upon each image and the red arrows indicated the time of ligating or reopening procedures.

The spatiotemporal evolution of the CBF over the imaged cortex before, during and after ischemia was illustrated in Fig. 4. Before the ligation of the middle branches of MCA, the direct communications (denoted by white arrows in Fig. 4 upper and left) of the branches in MCA is hard to determine because of the low flow and small diameter endowed with them. After ligation of the middle branch of MCA, the flow and diameter of the collaterals (denoted by white arrows in Fig. 4, second of the first row) from the adjacent branches increased significantly. As a consequence, the flow in the occluded branch (denoted by white triangle in Fig. 4) reappeared gradually through the supplying of the collaterals. However, the collateral flow from the anastomoses between MCA and posterior cerebral artery (PCA) was not determined in the acute phase of ischemia. The collateral flow also returned to the baseline level following the recirculation of the middle branch of MCA.

4. Discussion

In this study, laser speckle imaging with high spatiotemporal resolution was introduced to trace the evolution of collateral flow during acute focal ischemia located in the rat parietal cortex. The focal ischemia/reperfusion is induced by temporally occluding one of the branches of the MCA within the imaged cortex. Because of small-sized ischemia totally locating in the imaged area,[10] direct blood flow imaging technique, such as the laser speckle imaging,[6,12,13] could monitor the evolution of the collaterals around the ischemia with high spatial resolution and dynamic observing mode.

The individual variability of the anastomoses is responsible for the different outcomes of ischemic injury under clinical conditions of vascular occlusion. Larger and more numerous anastomoses will lead to smaller infarct within the vascular supplying territory.[2,14] More than two decades ago, Coyle had demonstrated the role of cerebral collaterals in restoring the blood flow to normal level in the territory of the occluded artery.[15] They found that the diameter and length of the cerebral collaterals were enhanced about 20 days after the occlusion of the MCA. There is also evidence for a delayed increase of CBF level in the border region of the ischemic territory as observed by laser Doppler flowmetry.[16]

The leptomeningeal anastomoses can be divided into 2 types according to their position and original source: inter-arterial and intra-arterial anastomoses.[17] The cortex is supplied by 3 main arteries: middle cerebral artery (MCA), anterior cerebral artery (ACA) and posterior cerebral artery (PCA). The inter-arterial anastomoses between 3 main arteries in the cortex include the MCA-ACA anastomoses, MCA-PCA anastomoses, and ACA-PCA anastomoses. The inter-arterial anastomoses connect the adjacent branches from the same common artery in the brain. In our study, we found that in the acute phase of ischemia, the intra-arterial anastomoses was immediately recruited to shunt the blood flow into the ischemic territory, whereas the inter-arterial anastomoses (PCA-MCA anastomoses) was not established within 1 hour after the ligation of one branch originating from MCA. This finding suggests the inter-anastomoses would be a potential therapeutic target when a sudden occlusion of the MCA was encountered, although the underlying mechanism is in need of further investigations.

Most of the previous studies concerning collateral anastomoses involved *in vitro* measurement of the vascular architectures[18] or disconnected flow status tracing by injecting exogenous contrast media repeatedly,[19] but these results lacked the dynamic evolution of the collateral time dimension. The conventional methods for dynamic monitoring of CBF, such as laser–Doppler flowmetry,[16] can provide information about CBF from one or several isolated points, while lacking spatial resolution. Scanning laser–Doppler can obtain spatially resolved relative CBF images, but the requirement of mechanical scanning limited both temporal and spatial resolution. Functional imaging techniques that are widely used in clinical studies recently, such as fMRI and PET[24] (Heiss *et al.*, 1994), can also provide three-dimension spatial maps of CBF, but acquire too limited temporal and spatial resolution to distinguish dynamic changes in leptomeningeal anastomoses. Therefore, a method that provides real-time spatially-resolved CBF images would further promote the understanding of collateral circulation after acute ischemia.

The difference in the response to focal ischemia in the cortex between intra- and inter-arterial anastomoses was well characterized in this study mainly due to the application of laser speckle imaging technique with high spatiotemporal resolution. Laser speckle imaging can get detailed cortical angioarchitecture as well as functional flow status *in vivo*.[6] LSI has been successfully applied to monitor the evolution of CBF in normal[7–9] and pathological conditions, such as cortical spreading depression[7,20] and ischemia,[7,21–23] due to its simplicity and high spatiotemporal resolution. The LSTCA method that advanced our previous works can provide even higher spatial resolution[12] and suppress the influence of speckle contrast from a stationary structure.[13] Therefore, with these new features, LSI may be useful to study functional collateral circulation and hence, guide therapy in ischemic disease.

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