PROGRESS IN NATURAL SCIENCE Vol. 13, No. 7, July 2003

Blood flow activation in rat somatosensory cortex under sciatic nerve stimulation revealed by laser speckle imaging*

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Received December 16, 2002; revised January 2, 2003

Abstract *In many functional neuroimaging research the change of local cerebral blood flow (CBF) induced by sensory stimulation is regarded as an indicator of the change in cortical neuronal activity although a precise and full spatio-temporal description of local CBF response coupled to neural activity has still not been laid out. Using the laser speckle imaging technique a relatively large exposed area in somatosensory cortex of rat was imaged for the observation of the variations of CBF during sciatic nerve stimulation. The results showed that cerebral blood flow activation was spatially localized and discretely distributed in the targeted microvasculature. Individual arteries, veins and capillaries in different diameters were activated with the time going. The response pattern of CBF related to the function of brain activity and energy metabolism is delineated exactly.*

Keywords: **blood flow activation, somatosensory cortex, sciatic nerve stimulation, laser speckle imaging.**

The postulation by Roy and Sherrington in 1890 that the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variation of functional activity provided a principal basis of neurophysiology and neuroenergetics for modern functional neuroimaging techniques[1]. The change in local cerebral blood flow (CBF) induced by sensory stimulation is considered as an index to investigate the effects of activated neural activity based on the above hypothesis. The response of evoked regional CBF to somatosensory stimulation in rats has been studied using some techniques such as Laser-Doppler flowmetry (LDF) and functional MRI (fMRI)[2,3]. In addition, quantitative and temporal relationship between regional CBF and neuronal activation has also been reported which combined electrophysiological and LDF techniques[4]. It is well-documented that CBF at the level of individual capillaries and the coupling of neuronal activity to flow in capillaries are fundamental aspects of homeostasis in the normal and the diseased brain[5]. However, it is hard for most present techniques to probe the dynamics of blood flow at this level due to their limitations of temporal or spatial resolution. Therefore a new alternative approach is needed to assess the intrinsic hemodynamic response in the corresponding cortical areas and elucidate the role of CBF in circulatory and metabolic correlates of functional activation in the brain[6].

Laser speckle imaging (LSI) technique can acquire the regional distribution of real-time velocity without the need of scan, which was proposed by Briers in the 1980's[7] and offered far better spatial and temporal resolution simultaneously. Recently it was introduced to characterize CBF behaviors with regard to neural mechanisms of brain events under the normal and pathophysiological conditions[8,9]. In this study, we applied LSI to describe the coupling relationship between CBF and neural activity based on our previous studies[10,11]. Changes of CBF in microvasculature were observed in a relatively large area through mapping contralateral somatosensory cortex under electrical stimulation of unilateral sciatic nerve. The response patterns of individual arteries, veins and capillaries which possessed diverse scales were differentiated in order to extract CBF activation at the individual vessel level. Furthermore, the reflection pattern like other intraoperative optical imaging systems endows it with broad prospect of clinical application[12]. It could furnish more fruitful information about CBF than LDF just from a limited number of isolated points[2,4], even though the resolution of scanning Laser-Doppler was still bound by the moving probe.

* Supported by the National Natural Science Foundation for Distinguished Young Scholars (Grant No. 60025514), and the National Natural Science Foundation of China (Grant No. 59836240)

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1 Materials and methods

1.1 Animal preparation

Male adult Sprage-Dawley rats weighing from 350 to 400 g ($n=16$) were anesthetized with an intraperitoneal injection of 2% α-chloralose and 10% urethane (50 and 600 mg/kg, respectively) to execute craniotomy. And atropine (0.4 mL/kg per hour) was administered to reduce mucous secretion during surgery. A closed cranial window (4×6 mm) over the parietal cortex was created as the following: A midline incision was made to expose the surface of the skull, then the skull overlying the hindlimb sensory cortex was bored to translucency with a dental drill under constant cooling with saline. The thinned skull preparation has the advantage over a full craniotomy since it keeps the dura mater intact and allows a long-term investigation into the changes in a somatosensory cortex within a single animal while preserving the integrity of the brain surface environment. The cranial window fully exposed the hindlimb sensory cortex in an area of 2.46×3.28 mm^2, centered at 2 mm caudal and 1.5 mm lateral to the bregma[13,14].

The animals were mounted in a stereotaxic frame, and body temperature was maintained at (37.0 ± 0.5) ℃ with a thermostatic heating blanket. A tracheotomy was performed to enable a mechanical ventilation using a ventilator (TKR-200C, Animal Mechanical Respirator, China) with a mixture of air and oxygen (20% O_2, 80% N_2) to achieve physiological arterial blood levels of O_2 and CO_2 tension. The right femoral artery and vein were cannulated for measurement of blood pressure (PcLab Instruments, China) and intravenous administration of drugs. Periodically, a small volume of blood was drawn from the femoral artery, and the blood gas pressure (PaO_2, $PaCO_2$) and pH value were analyzed (JBP-607, Dissolved Oxygen Analyzer, China). After surgery, the animals were left for at least half an hour before the experiment began and supplemental doses of anesthetic (one-fifth initial dose/h) were also needed.

1.2 LSI system

The LSI system composed of a stereo microscopy (SZ6045TR, Olympus, Japan) and a CCD camera (Pixelfly, PCO Computer Optics, Germany) is shown in Fig. 1. The light beam from a He-Ne laser ($\lambda=632.8$ nm, 3 mW, its coherence length approximates to 1 cm) is coupled into an 8 mm diameter fiber, which is adjusted to illuminate the area of interest evenly. Raw speckle images with 480×640 pixels are acquired through the computer at 40 Hz and the illuminated area is dependent of the magnification. This system offers a high spatial resolution (25 ms) and a high temporal resolution (13 μm), with the discrimination of 9% change of velocity[15].

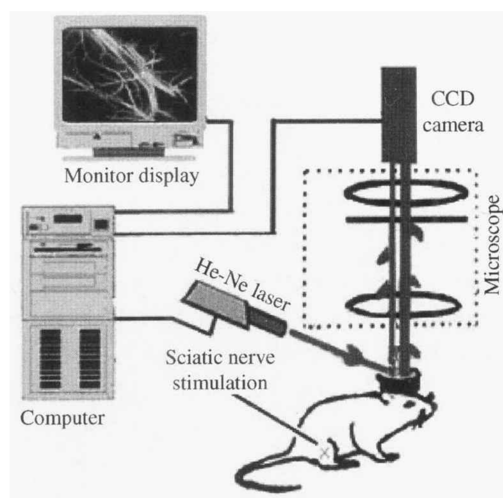

Fig. 1. Schematic of system for laser speckle imaging.

1.3 Sciatic nerve stimulation

Stimulation of the sciatic nerve was similar to that used in conventional physiological studies[14,16]. The contralateral sciatic nerve was dissected free and cut proximal to the bifurcation into the tibial and peroneal nerves. Then the proximal end was placed on a pair of silver electrodes and bathed in a pool of warm mineral oil in order to keep wettish. We stimulated the single sciatic nerve on the left with a rectangular pulse of 350 mV intensity, 0.5 ms duration, and at a rate of 5 Hz frequency for a train duration of 2 s (Multi Channel Systems, Germany). These parameters were chosen to optimize vessel response without affecting systemic blood pressure and kept constant during each experiment. In all animals, a single-trial procedure was preformed 15 to 20 times and separated by an interval of at least 4 min. A total of 400 frames of raw images were obtained in one 10-second single-trial while the electrical stimuli started at the 2nd second since the images in the first two seconds were recorded as the baseline. Notably, here the data acquisition was synchronized with the electrical signal via an appropriate trigger circuit, and therefore the procedures of data analysis described below could improve the reproducibility of our results and enhance

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the signal-to-noise ratio. All recorded data were finished within 3~4 h after the beginning of chloralose-urethane anesthesia.

1.4 Data processing and analysis

Each of LSI digital images is a data matrix of 640×480, which corresponds to a field of $2.46 \times 3.28\ mm^2$ of the CCD camera, yielding an in-plane resolution of $27 \times 27\ \mu m^2$. In our system, the pixel size is $9.9 \times 9.9\ \mu m^2$ and the integration time, T, for each image is 25 ms. As a full-field laser speckle technique, LSI exploits the spatial statistics properties of laser speckle to obtain the two-dimensional velocity distribution with a high spatial and temporal resolution through analyzing the spatial blurring of the speckle image obtained by CCD camera. This blurring is represented as the local speckle contrast K, which is defined as the ratio of the standard deviation to the mean intensity; the link between the speckle contrast and the correlation time can be manifested by the equation $K = \left[\frac{\tau_c}{2T}\{1-\exp(-2T/\tau_c)\}\right]$, where the correlation time, τ_c, is given by $\tau_c = 1/(ak_0 v)$, and k_0 is the light wave number, and a is a factor that depends on the Lorentzian width and scattering properties of the tissue. The value of τ_c is assumed to be inversely proportional to flow velocity and can be used to compute the relative velocity[7,8]. The relative change of blood flow is measured by LSI, which is the same as LDF. The LDF signal reflects a Doppler shift caused by all the moving scatters when they interact with photons within the penetration depth of laser. It is also subject to biological properties of tissues and represents the relative change of flow[17]. The detailed algorithm of conversion of raw speckle images into relative blood-flow images is available in Ref. [10]. The average strategy is effective to suppress physiological noises and minimize effects of systemic physiological factors (heart-beat, breath, blood pressure and spontaneous oscillations of pulsatile blood). Each set of 5 speckle contrast images obtained in each single trial was averaged together before further analysis[18].

2 Results

With LSI technique we monitored blood flow in somatosensory cortex in a total of 16 rats under electrical stimulation of sciatic nerve, and obtained the activated blood flow distribution at different levels of arteries/veins and the change of activated areas. Although there existed slight differences in individual anatomic features in the rat cortex, we could eliminate this influence since the imaged area was much bigger than the scope demarcated by Hall et al.[13] One example of our results is shown in Fig. 2, in which the brighter areas correspond to the area of increased blood flow. In comparison with LDF, an area of $1\ mm^2$ ROI in Fig. 2 (a) was chosen to evaluate its mean velocity (Fig. 3): the evoked CBF started to increase (0.7 ± 0.1) s, peaked at (3.1 ± 0.2) s and then returned to the baseline level. It is coherent with the conclusions obtained from LDF technique[2,4]. In order to differentiate the response patterns of artery/vein under the same stimulus, we labeled six distinct levels of vessels in Fig. 2 (a) and displayed their changes of blood flow. The results clearly showed that the response patterns of arteries and veins in the somatosensory cortex were totally different: vein 1 (V-1, ~140 μm in diameter) almost remained unaffected, and arteriole 1 (A-1, ~35 μm in diameter) responded slowly; arteriole 2 (A-2, ~35 μm in diameter) peaked at (3.5 ± 0.5) s after the onset of stimulation and then reached the steady-state plateau, and vein 2 (V-2, ~70 μm in diameter) presented a delay and mild response; blood flow in the capillaries (A-3 and V-3, ~10 μm in diameter) surged readily and increased significantly. We also measured the changes in arteries and veins with different diameters and the results are shown in Fig. 4. Our statistical results exhibited that arterioles (A-II, ~35 μm in diameter) dilated abruptly ($p<0.05$) but arteriole 1 (A-I) did not change and dilated slightly at 5~6 s after the end of stimulation ($p<0.05$). No alterations in vein with diameter of >70 μm were observed during sciatic nerve stimulation ($p>0.05$). We found that the blood flow in capillaries in hindlimb sensory cortex was firstly activated to increase at (0.5 ± 0.2) s; then arterioles with diameter of >70 μm began to respond at (2.5 ± 0.5) s, dilated up to maximum at (3.5 ± 0.5) s and came back to the prestimulus level; and finally the activation propagated to the entire scope of somatosensory cortex. Blood flow in arteriole 1 did not increase until after 5~6 s end of stimulation since it was situated farther from the hindlimb cortex. The activation pattern of cerebral blood flow is discrete in spatial distribution and highly localized in the evoked cortex with the temporal evolution. This is consistent with the hypothesis of Roy and Sherrington and the conclusions drawn by other research groups[2~4,14].

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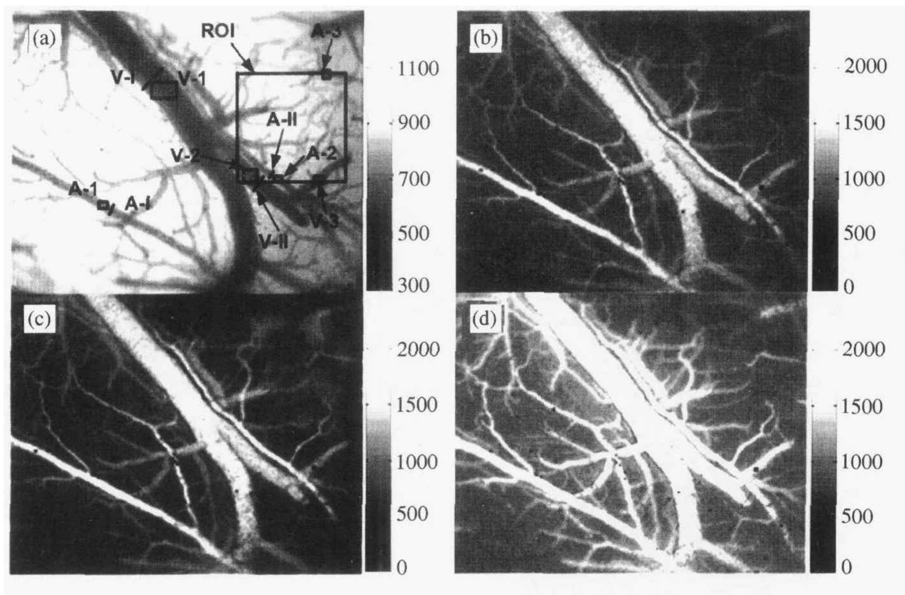

Fig. 2. Blood flow change in contralateral somatosensory cortex of rats under unilateral sciatic nerve stimulation. (a) A vascular topography illuminated with green light (540 ± 20 nm); (b) ~ (d) blood activation map at prestimulus, 1 s and 3 s after the onset of stimulation (the relative blood-flow images are shown and converted from the speckle-contrast images, in which the brighter areas correspond to the area of increased blood flow.), respectively. A-1, 2, 3 and V-1, 2, 3 represent the arbitrarily selected regions-of-interest for monitoring changes in blood flow. A-I, II and V-I, II represent the selected loci on the vessel whose diameters are measured in the experiment.

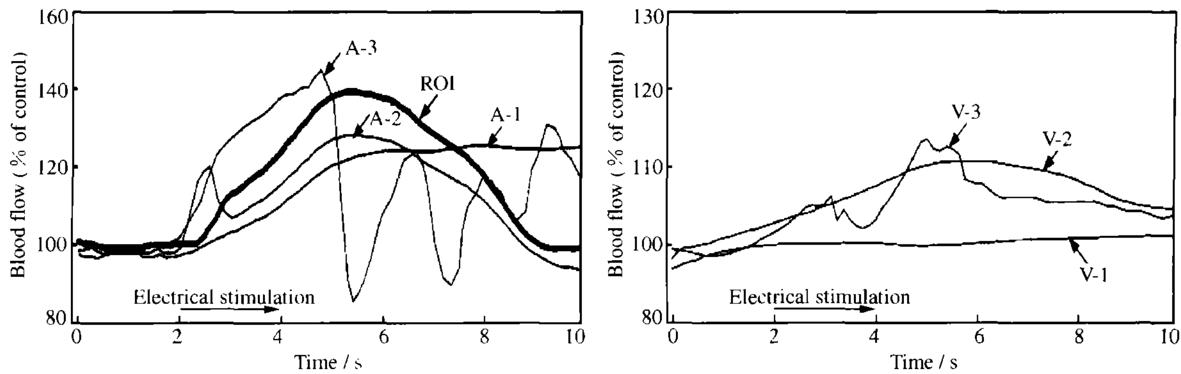

Fig. 3. The relative change of blood flow in 6 areas indicated in Fig. 2 (a) (divided by the values of prestimulus).

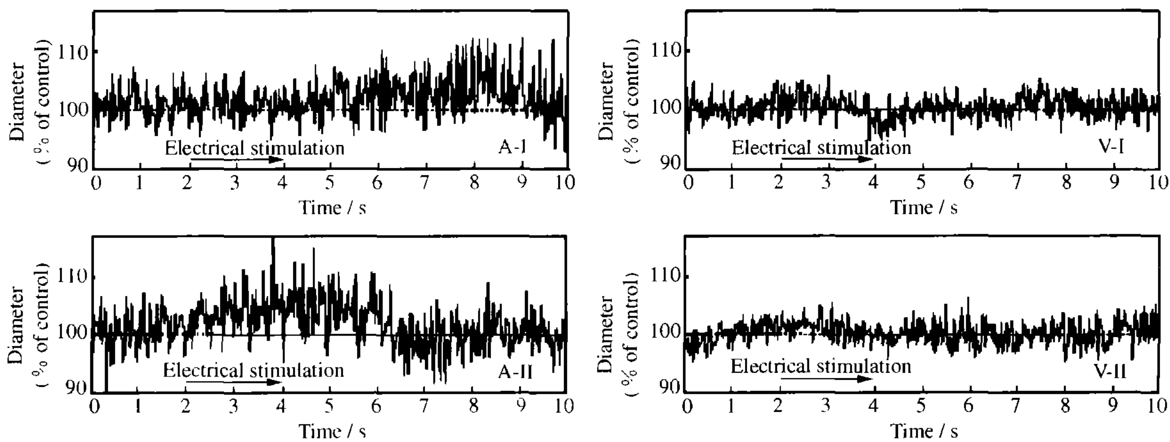

Fig. 4. Relative alterations in vessel diameter during sciatic nerve stimulation (divided by the values of prestimulus).

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3 Discussion

The present study is influenced by various kinds of experimental conditions due to the complicacy of biological experiments. The first factor is stimulation parameters that should reach the threshold of response, and not affect systemic blood pressure and evoke the maximal magnitude of vascular response. The optimal parameters (350 mV, 0.5 ms, and 5 Hz) were adopted in our experiments. The second is anaesthesia condition of animals. It was proved by the former investigations that chloralose-urethane is most suitable for the study on neurovascular coupling since it induces minimal cardiovascular effects[4,14]. In order to minimize spontaneous oscillations (also known as "vasomotion"), it is important to maintain an adequate anesthesia and keep blood pressure above 80 mmHg. Anesthesia would exert direct influence on the animals' respiration and probably cause CBF fluctuating in nearly synchrony with the respiratory cycle. Thirdly, tissue pH and blood composition also influence regional CBF. Acids and bases cause cerebral vasodilation and vasoconstriction, respectively. When functional activity in blood-perfused tissue is activated, the rate of energy metabolism is increased and the gas tension of CO_2 is elevated to cause cerebral vasodilation and increase local CBF. Electrical stimulation of unilateral sciatic nerve is a classical biological model which was used to probe effects of increased functional activity in somatosensory cortex[2,14,16]. In our experiments, several physiological parameters (including body temperature, femoral blood pressure, PaO_2, $PaCO_2$ and pH) were monitored for keeping a normal physiological status during the experiment. Thus it improved the reliability and reproducibility of our results.

Until now the response of evoked CBF to somatosensory stimulation in rats has been studied using some techniques such as LDF[2] and fMRI[3] under laboratory or clinical conditions. However, those conventional methods have their own limitations like lower temporal /spatial resolutions in fMRI, or radioactive effects in PET, or limited information from isolated points in LDF. It is difficult to apprehend comprehensively behaviors of CBF during brain functional activity. It is almost certain that the dynamic regulation of the cerebral circulation is not mediated by a single exclusive mechanism but is achieved by numerous factors acting in concert. Most of the effects of these neural vasomotor pathways were observed in pial arteries and might not apply to the small parenchymal resistance vessels that regulate the blood flow, which is also known as the "spatial heterogeneity of microcirculation"[1]. The sample tissue volume of conventional LDF technique is approximately 1 mm^3[2,4,18], as exerted great limitations on its application[17].

Here LSI technique we present provides a new alternative approach in measurement of blood flow. It develops the spatial statistical characteristics of time-varying image speckle, extracts the velocity information from speckle signals within an area of 5×5 pixels and obtains the velocity distribution in the whole region. Its spatial resolution is equal to the area in the image plane corresponding to 5×5 pixels, far better than LDF[15]. LSI is capable of accurately imaging the cortical blood flow response over an area ranging from a few millimeters to a centimeter over time scales of milliseconds to hours. For its higher temporal and spatial resolution (here 25 ms and 27 μm, respectively), we can choose small regions-of-interest in two-dimensional maps of blood vessels so as to analyze the spatial patterns of different vessels response to sciatic nerve stimulation and also show their evolution along the time axis, as can furnish more information to characterize the regulation mechanism of microcirculation associated with cerebral functional events. The finding of this study is that spatial response of CBF is highly localized in cortical anatomic distribution and discretely coupled to the microvasculature in the targeted cortex. Different levels of arteries, veins and capillaries are activated successively with the time varying. Compared with the former conclusions, we found more elaborated details besides those accordant results and offered a new proof to the hypothesis proposed by Roy and Sherrington more than 100 years ago.

Acknowledgements The authors thank Chen Shangbin, Dr. Li Pengcheng, and Dr. Zeng Shaoqun for their help in the experiment, Zhang Wei and Cen Jian for their assistance in handling the instrument. The authors particularly thank Dr. Ngai, A. C., Dr. Bandettini, P. and Dr. Sokoloff, L. for their invaluable help during the preparation of this manuscript.

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