Measurement of Distribution of Blood-flow Change in Exposed Cortex by Laser Speckle Flowgraphy

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The change in the cortical blood flow of guinea pigs during auditory stimulation is measured laser speckle flowgraphy (LSFG). Accuracy of LSFG depends on the number of the frames the blood flow calculation. The blood-flow maps of rotating disks and cortical tissues are calculated from various numbers of frames of speckle pattern, and the influence of the statistical error and averaging effect on the results is evaluated to optimise the LSFG system. The blood-flow map obtained from successive 24–30 frames can appropriately identify the change in the blood flow caused by auditory stimulation.

Key Words: Laser, Speckle, Blood flow, Imaging, Brain activation

1. Introduction

The functional activation of the brain induces the local changes in the blood flow in the cortical tissue. The image analysis of the exposed cortex to measure the optical intrinsic signals has enabled us to visualise the area of the brain activation. Laser Doppler flowmeter (LDF) has been applied to measure the blood flow in the tissue. However LDF can normally measure the blood flow at a point. Laser speckle flowgraphy (LSFG)[1,2] has been employed to obtain the two-dimensional distribution of the blood flow in tissues. LSFG is used in medical field such as ophthalmologic. We applied LSFG to measure the blood flow in the cortical tissue of guinea pigs. When the tissue irradiated by laser light is imaged on a CCD camera, a random interference pattern, speckle pattern, can be observed. In the LSFG system, the distribution of the mean blur rate (MBR) is calculated from the spatial and temporal intensity changes in the speckle pattern as the blood-flow map. Since the MBR value includes a statistical error, it is necessary to average several frames of the speckle pattern to minimise the error. However, the large number of the frames for the MBR calculation reduces the temporal resolution by the averaging effect. It is important to determine the appropriate number of frames to calculate the MBR value.

In this study, the change in the cortical blood flow of guinea pigs during auditory stimulation was measured by LSFG. The measured MBR value reflects both the fluctuation of the blood flow and the statistical error. The speed of a rotating disk was also measured to evaluate the effect of the statistical error. The appropriate number of frames for calculating the blood flow in the cortical tissue of guinea pigs by LSFG was determined.

2. Measurements by Laser Speckle Flowgraphy

The laser speckle system was installed in a stereomicroscope as shown in Fig.1. The object was irradiated with a near-infrared laser diode at 780-nm wavelength. The diffusely reflected light from the object was detected with a monochrome CCD camera (400 × 400 pixels). The speckle pattern was acquired every 1/30 seconds.

The objects were a plastic plate and the exposed cortex of guinea pigs. The plastic plate was rotated by a motor at the constant speed. In the blood-flow measurements, guinea pigs were anaesthetised and the skull was removed to observe the brain cortex. The exposed cortex was covered with physiologic saline solution. The change in blood flow caused by the brain activation induced by auditory stimulation was measured by LSFG. One measurement cycle consisted of a 3-second resting period, a 1-second auditory stimulation period and a 6-second resting period. The measurement cycles were repeated 20 times to calculate the average of these

Fig.1 LSFG system and experimental setup. Laser light at 780 nm wavelength is reflected by a mirror and irradiates the object.

repetitions. The interval of each measurement cycle was 20 seconds.

The spatial and temporal intensity changes in the speckle pattern depend on the speed of red blood cells in the cortical tissue. The blur of the recorded speckle pattern increases with an increase in speed of the blood flow. The mean blur rate (MBR) was defined to quantify the blur of the speckle pattern corresponding to the blood flow. The MBR value is calculated from several frames of the speckle pattern. In the case where the MBR value is calculated from 3 frames, 2 × 2 pixels in three successive speckle images are used to calculate a MBR value in the MBR map (Fig. 2). The detail of the algorithm to calculate MBR value from the successive frames is described elsewhere. [1] The number of the flames to calculate the blood-flow map was varied from 6 to 99 in order to evaluate the statistical error and averaging effect. The blood-flow maps were calculated for every 0.2 seconds. The change in blood flow ΔMBR_i was calculated from the MBR value during the rest state (MBR_0) and that at time i (MBR_i).

$$\Delta MBR_i = [(MBR_i - MBR_0)/MBR_0] \cdot 100 \quad (1)$$

3. Results

The speed of the rotating disk was measured by LSFG. The speed was varied from 8 rpm to 120 rpm. Figure 3 shows the relationship between the speed of the rotating disk and the MBR value measured by LSFG. The MBR value proportionally increases with an increase in speed of the rotating disk. Figure 4 shows the distribution of MBR value (MBR map) of the rotating disk. Since the disk was rotated at the constant speed, the spatial fluctuation of MBR value was caused by the statistical noise. The statistical noise is distinctly observed in the MBR map calculated from 6 frames of speckle patterns as shown in Fig. 4(a). The statistical noise decreases with an increase in the number of frames for MBR calculation. The statistical noise in the MBR map calculated from 30 frames is almost the same as that calculated from 99 frames.

The images of the exposed cortex of a guinea pig illuminated with a halogen lamp and with the laser diode are shown in Figs. 5(a) and 5(b), respectively. Speckle pattern can be observed in Fig. 5(b). The intensity distribution of the speckle pattern in Fig. 5(b) was represented by colour scale.

Figs. 6(a), 6(b) and 6(c) show the distributions of the MBR values (blood-flow map) calculated from 6, 30 and 99 successive frames of the speckle pattern, respectively. Greater MBR values are observed along the blood vessels. The spatial fluctuation of the MBR value which is similar to the statistical noise shown in Fig. 4(a) can be observed in the MBR map calculated from 6 frames. Since a blood flow in unobserved capillary bed is also detected in the blood-flow map of the cortical tissue, it is difficult to distinguish the statistical noise from blood flow in unobserved capillary bed. The spatial fluctuations of the MBR value in the MBR maps calculated from 30 and 99 frames are obviously smaller than that calculated from 6 frames.

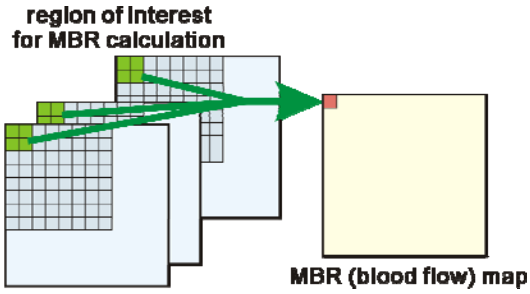

Fig.2 Schematic diagram of the calculation of the mean blue rate (MBR).

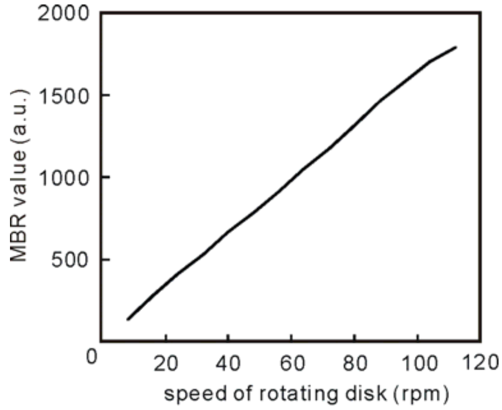

Fig.3 The relationship between the MBR value and the speed of the rotating disk.

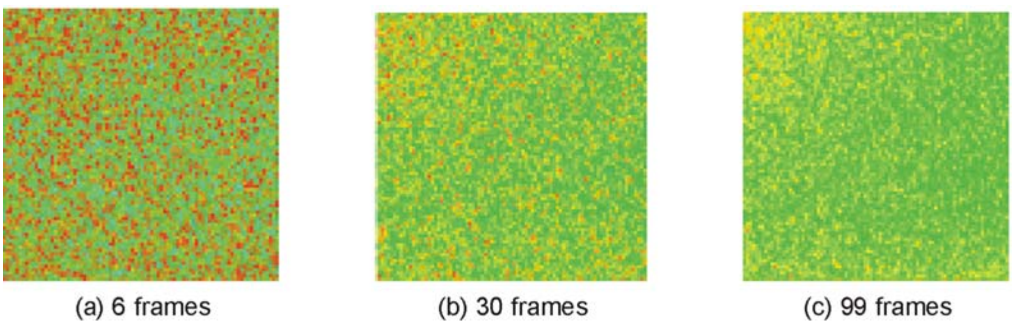

Fig.4 The distribution of MBR value (MBR map) of the rotating disk. Number of frames for MBR-value calculation is varied from 6 to 99 frames.

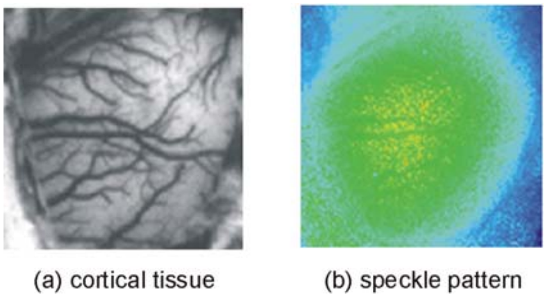

Fig.5 The blood-flow map of the cortical tissue of a guinea pig measured by laser speckle flowgraphy.

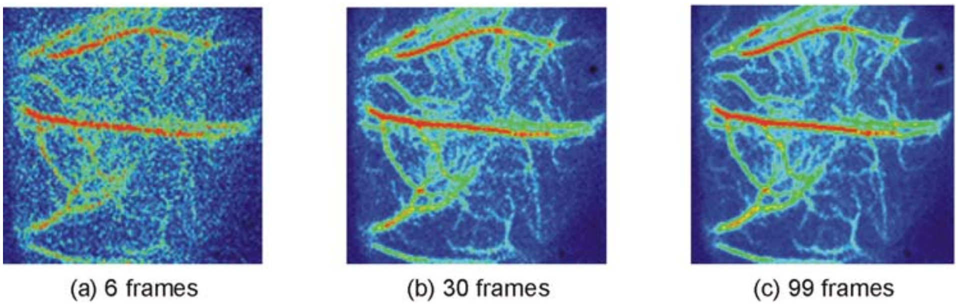

Fig. 6 The dependence of statistical noise in MBR-maps on the number of frames for the calculation. The number of the frames for the blood-flow map calculation is varied from 6 to 99.

The mean MBR values of the MBR maps of the rotating disk and the cortical blood flow were calculated from various numbers of frames for evaluating the effect of the number of frames in the MBR calculation on the statistical noise. The results are shown in Fig. 7. The MBR values are normalised by the MBR value obtained from 99 frames of the speckle pattern. The relationship between the number of frames and the mean MBR value for the cortical blood flow is almost the same as that for the rotating disk. The mean MBR value steeply increases with a decrease in the number of the frames for the MBR calculation when the number of the frames is less than 20. This increase in the mean MBR value is caused by the statistical noise. It should be noted that the mean MBR value for both the rotating disk and the cortical blood flow is almost constant when the number of the frames for the MBR calculation is greater than 30. These results indicate that the influence of the statistical noise can be neglected if the MBR map is calculated from more than 30 frames of the speckle pattern.

The temporal changes in the MBR value (blood flow) calculated from 6, 30 and 96 frames of the speckle pattern of the cortical tissue are shown in Fig. 8. The blood flow steeply increases just after the onset of auditory stimulation. The blood-flow calculated from 6 frames constantly fluctuates and this fluctuation is mainly caused by the statistical error. This type fluctuation cannot be observed in the results calculated from 30 and 96 frames. The change in the MBR value caused by auditory stimulation calculated from 96 frames is obviously smaller than that calculated from 30 frames. This underestimate of the change in MBR value is caused by the averaging effect. These results indicate that the optimal number of frames for the calculation of the MBR map of the cortical blood flow to minimise both the statistical error and

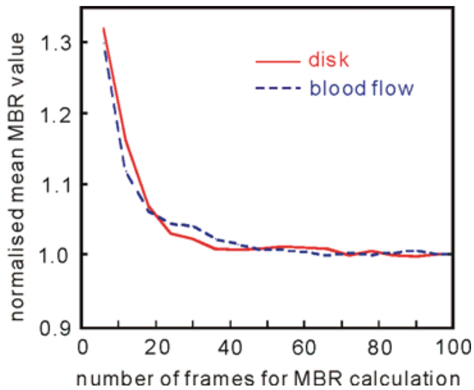

Fig. 7 The relationship between the mean MBR value of the rotating disk and blood flow measured by LSFG and the number of flames for the calculation.

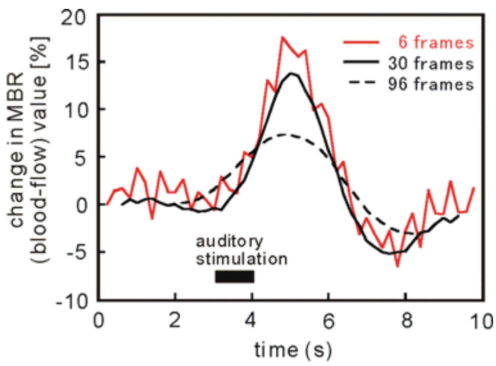

Fig. 8 The change in blood flow in the cortical tissue of a guinea pig during auditory stimulation.

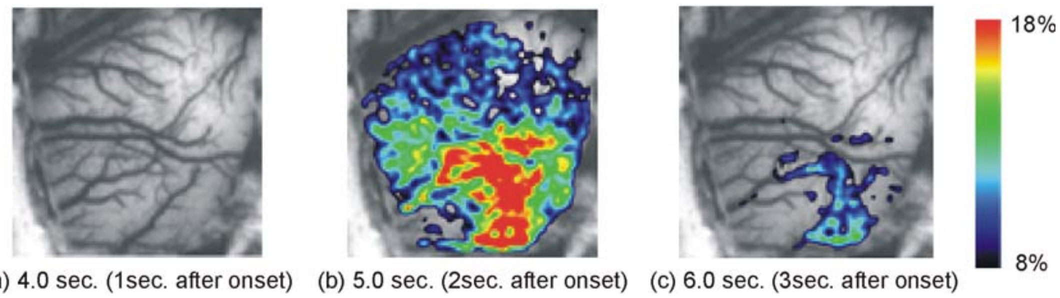

Fig. 9 The distribution of the change in the blood-flow cause by auditory stimulation calculated from 30 frames.

averaging effect is 30 frames. The blood-flow maps are obtained for every 0.2 seconds. The MBR values in the blood-flow maps are averaged for 1 second when the number of frames to calculate the MBR value is 30 frames.

The distributions of the change in the blood flow at 1, 2 and 3 seconds after the onset of auditory stimulation are shown in Figs. 9(a), 9(b) and 9(c), respectively. The MBR map was calculated from 30 frames of the speckle pattern. The MBR values greater than 8% are superimposed on the image of the cortical tissue as the activated area. The blood flow in the broad area was changed by the auditory stimulation.

5. Conclusions

The change in the blood flow in the cortical tissue of guinea pigs caused by auditory stimulation was measured by laser speckle flowgraphy (LSFG). The relationship between the statistical error in a MBR map and the number of frames for the MBR calculation is analysed by the experiments using a rotating disk to optimise the number of frames for the MBR calculation. The statistical noise in the MBR map of the rotating disk decreases with an increase in the number of frames. The tendency of the statistical noise in the MBR map of the cortical blood flow was almost the same as that of the rotating disk. The results of LSFG measurements of the rotating disk and the cortical blood flow indicate that the optimal number of frame to minimise the statistical noise and averaging effect is about 30 frames.

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