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# CBF Change Evoked by Somatosensory Activation Measured by Laser-Doppler Flowmetry: Independent Evaluation of RBC Velocity and RBC Concentration

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**Abstract:** The purpose of this study was to examine the timing and magnitude of cerebral blood flow (CBF) responses to neuronal activation. We measured the changes in local CBF (LCBF), red blood cell (RBC) velocity and RBC concentration by laser-Doppler flowmetry (LDF) as well as field potential recordings during activation of the somatosensory cortex of the rat in response to electrical stimulation of the hind paw. Electrical stimuli, 0.1 ms pulses of 1–1.5 mA for 5 s, were applied at 0.2, 0.5, 5, 10 and 50 Hz under α-chloralose anesthesia. LCBF showed the maximum increase at 5 Hz, and rose approximately 0.5 s after the onset of stimulation regardless of the frequency. The maximum frequency of the field potentials was also obtained at 5 Hz. During activation of the somatosensory cortex, the onset of rise in RBC concentration did not precede that of RBC velocity, and the peak RBC concentration was noted earlier than that of both LCBF and RBC velocity, suggesting that both arteriolar diameter and active changes in the capillary contributed to the LCBF response. [Japanese Journal of Physiology, 49, 289–296, 1999]

*Key words*: field potential, functional activation, hind paw stimulation, laser-Doppler flowmetry, local cerebral blood flow.

The mechanism of coupling between neuronal activation and blood flow still remains to be elucidated despite the fact that numerous investigations were conducted over the past few decades. We focus, in this study, on the mechanism of regulation of the early changes in cerebral blood flow (CBF) following neuronal activation. We examine which vessel (i.e., the arteriole, capillary or venule) primarily regulates the changes in local CBF (LCBF). Previous studies suggest that the diameter of the pial arteriole determines the cortical blood flow during neuronal activation of the cortex [1]. Recently, Malonek and Grinvald [2] observed, in the cat visual cortex, that changes in the total hemoglobin content (corresponding to cerebral blood volume) preceded those in the oxyhemoglobin concentration (with resultant increase in CBF). Their observation suggests that the capillary changes occur prior to arteriolar dilatation.

The purpose of our current study was to investigate the regulatory mechanisms of the change in LCBF evoked by neuronal activation using laser-Doppler flowmetry (LDF). Cerebral microcirculation is often monitored by LDF, and a number of laboratories have used LDF to demonstrate the changes in CBF related to cerebral activation in response to various stimuli [1, 3–6]. On the other hand, there have been only a few attempts to observe the hemodynamics of red blood cell (RBC) velocity and RBC concentration independently, although validation of these parameters was achieved by an *in vitro* experiment [4]. Because these

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parameters are affected by the high level of noise in *in vivo* studies, we accumulated multiple data by repeated triggering in order to reduce the noise level. We then examined the relationship between the changes in these hemodynamic parameters during neuronal activation in response to hind paw electrical stimulation.

Another objective of this study was to estimate the relationship between neuronal activity and the changes in LCBF. Although a relationship between the stimulus frequency and CBF response has been reported [7, 8], these experiments evaluated the amplitude of neuronal activity using surface electrodes on the skull. Such evaluation inevitably has limitations for the accurate estimation of neuronal activity. In contrast, in this study, we recorded the field potential of a local area using an electrode inserted into the cortex, and evaluated the relationship between this recording and LDF parameters.

This is a report of the independent evaluation of the *in vivo* dynamics of RBC velocity and RBC concentration during sensory stimulation using LDF. Such data, namely of change in the LCBF during neuronal activation, are of potential value for a better understanding of brain imaging techniques.

## MATERIALS AND METHODS

**Animal preparation.** Male Sprague-Dawley rats (300–380 g) were anesthetized with halothane (4% induction and 1.5% during surgery) in 30% $O_2$ and 70% $N_2O$. The tail artery was cannulated for monitoring blood pressure and blood gas sampling, and the left femoral vein was cannulated for intravenous drug administration. Following tracheotomy, α-chloralose (100 mg $kg^{-1}$, I.V.) was administered, and halothane and nitrous oxide were discontinued. The rats were then immobilized with pancuronium bromide (0.7 mg $kg^{-1}$, I.V.), and mechanically ventilated with a respirator (SN-480-7, Shinano, Japan) throughout the experimental period. Anesthesia was maintained with α-chloralose (35 mg $kg^{-1}$ $h^{-1}$, I.V.) and muscle relaxation with pancuronium bromide (0.8 mg $kg^{-1}$ $h^{-1}$, I.V.). Body temperature was maintained at 37.5±0.2 °C with the aid of a heating pad (ATC-101, Unique Medical, Japan). Arterial blood was sampled from the tail artery for blood gas analysis three times throughout the experiment; before, during and just after the measurements. Arterial blood pressure was monitored during the experiment, and the mean value (MABP) was calculated from the average of measurements taken at the three aforementioned time points. The rats were fixed in a stereotactic frame, and the parietal bone was thinned to translucency at the left somatosensory cortex, over an area of 3×3 mm, centered 2.5 mm caudal and 2.5 mm lateral to the bregma.

**LDF measurement.** LCBF was measured with an LDF (Ne-He, wavelength of 780 nm, maximal intensity of 0.8 mW; Periflux 4001 Master, Perimed, Sweden) and an LDF probe with a tip diameter of 0.46 mm (Probe 411, Perimed, Sweden). LDF measures blood flow based on the Doppler effect with laser light. The frequency shift of the scattered radiation is caused by RBCs moving in the blood vessels. The amount by which the frequency is shifted by the Doppler effect corresponds to the RBC concentration, and the average shift in frequency is related to RBC velocity. Our LDF system simultaneously provided three parameters; flux (=LCBF), RBC velocity and RBC concentration, where RBC velocity=LCBF/RBC concentration [9]. The area of LDF measurement has been reported to be about 1 $mm^3$ [10, 11]. It has been confirmed that RBC behavior in the capillary is the main contributor to the LDF signals [4]. A time constant of 0.03 s was used for all LDF signals (LCBF, RBC velocity and RBC concentration). The LDF probe was located on the somatosensory area of the hind paw, perpendicular to the brain surface. It was attached to the thinned skull and then fine-positioned for maximum signal change during stimulation, avoiding areas with large blood vessels.

**Hind paw stimulation.** Activation of the cortex was carried out by electrical stimulation of the hind paw with rectangular pulses (0.1 ms) through a pair of small needle electrodes inserted under the skin of the right hind paw. Stimulation was carried out for about 1 h after preparation. In all rats, a current stimulus of 1 to 1.5 mA, which elicited a change in LCBF, was applied at a frequency of 5 Hz for a duration of 5 s. In the experiments for the analysis of frequency dependency (study 1), we used 9 rats and varied the frequency (0.2, 0.5, 5, 10 and 50 Hz) of the electrical stimuli for 5-s durations at a selected intensity. The order of the stimulus frequencies was selected randomly; at each stimulus frequency, 20 successive stimuli were applied at 80-s intervals. In the experiments performed for determination of the time-course of change in RBC velocity and RBC concentration (study 2), we used 14 rats and applied electrical stimuli of a selected intensity at 5 Hz for a duration of 5 s; 50 successive stimuli were applied at 80-s intervals. Blank sampling of LDF signals, without electrical stimulation, was performed after the application of each set of stimuli. Data acquisition of LDF was synchronized to the respirator cycle.

**Electrophysiology.** After LDF measurements,

we recorded the field potentials to confirm that the LDF probe was indeed located at the activation area. A tungsten microelectrode (12 MΩ), for recording the field potentials, was inserted into the cortex through the thinned portion of the skull at an angle of 45° relative to the LDF probe. The tip of the electrode was set at a depth of about 0.5 mm from the surface of the cortex, just beneath the LDF probe. An Ag–AgCl indifferent electrode was placed between the skull bone and the scalp. In 6 of the 9 rats in study 1, the field potentials in response to various stimulus frequencies were also recorded to estimate the correlation between change in LCBF and neuronal activation.

**Data analysis.** Data from the successive measurements of the LDF signals, field potentials and arterial blood pressure values were accumulated by using MacLab data acquisition software (AD Instruments, Australia), digitized at 40 Hz, and saved on a disk for off-line analysis. The LDF data were normalized to the blank. The rise-time of the LDF signal was determined as the time at the intersection of the baseline (pre-stimulus level) by the extrapolated line drawn on the normalized response curve from 90 to 10% of the peak. The termination-time was also determined as the time of the intersection of the baseline by a similarly extrapolated line (Fig. 1). The peak-time was the time at which the response curve reached the maximum height. The response magnitude was calculated as an integral of the response curve from the rise-time to termination-time, and was considered to reflect the total amount of increase in blood flow. LDF data which included spontaneous oscillations in the pre-stimulus period larger than the evoked response were excluded from the analysis. In the field potential analysis, the number of spike-shaped potentials which exceeded the noise level during the stimulus period was counted.

In study 1, parameters (i.e. rise-time, peak-time, termination-time and magnitude) among each stimulus frequency were statistically analyzed by ANOVA (repeated measurements) and multiple comparisons (Bonferroni). In study 2, significant differences between the rise-time, peak-time and termination-time of RBC velocity and RBC concentration were statistically examined using the Wilcoxon test.

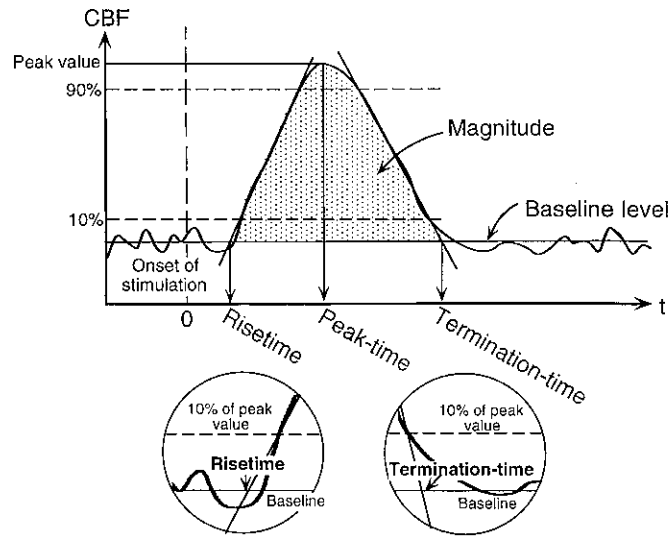


**Fig. 1. Schematic diagram illustrating the calculation method of the time parameters and response magnitude.** The rise-time was determined as the time at the intersection of the baseline (pre-stimulus level) by the extrapolated line drawn on the normalized response curve from 90 to 10% of the peak, the termination-time was also determined as the time at the intersection of the baseline by a similar extrapolated line. The peak-time was the time at which the response curve reached the maximum height. The response magnitude was calculated as an integral of the response curve from the rise-time to the termination-time.

## RESULTS

The mean arterial blood pressure (MABP) was 109±9 mm Hg (mean±SD, $n$=23). MABP was stable throughout the experiment and did not change significantly during stimulation (Fig. 2). The blood gas values were as follows; $PaO_2$=108±13 mmHg, $PaCO_2$=34.7±2.5 mmHg and pH=7.43±0.04 ($n$=23). These values were maintained within a stable range by regulating the stroke volume and oxygen concentration of the inspired gas.

### Characteristics of changes in field potentials in response to stimulus frequency

At frequencies≤5 Hz, each electrical stimulus evoked a corresponding field potential. At frequencies≥10 Hz, the field potentials did not correspond to the electrical stimulation pulses except for the first one (Fig. 2 and Table 1), implying that the field potentials recorded in response to current pulses subsequent to the first pulse were either markedly decreased in amplitude or entirely absent at stimulus frequencies≥10 Hz. On the other hand, the latency of onset of the first potential after stimulation was consistent for all stimulus frequencies, and was 10.1±0.7 ms.

### LCBF with change in stimulus frequency

The rise-time, peak-time and termination-time of the LCBF response curve were measured as a function of the stimulus frequency (study 1). The rise-time was nearly constant, at approximately 0.5 s, regardless of the stimulus frequency, between 0.2 and 50 Hz (NS,

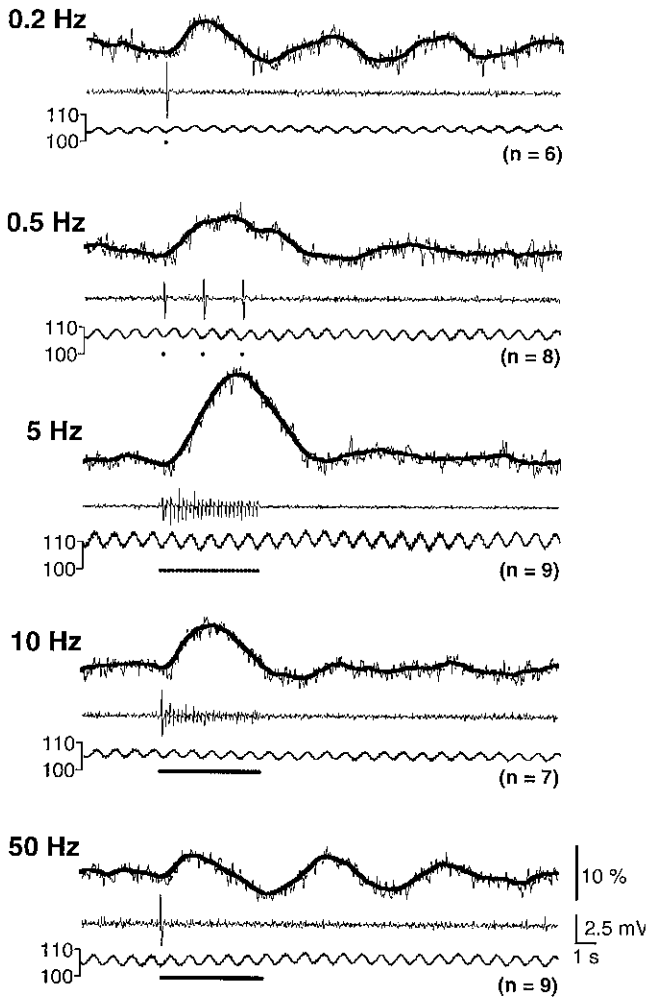


**Fig. 2. Time-course of changes in LCBF, cortical activation and arterial blood pressure responses for various frequencies of 5-s stimulation of the hind paw.** Each data set consists of the changes in LCBF (**top**), field potentials (**2nd**), arterial blood pressure values (mmHg, **3rd**) and the mark of electrical pulse (**bottom**). LCBF change was normalized to blank stimulation, and averaged by the number of animals used. Representative field potential and blood pressure recordings show a sample of the recording of one animal. Note that the frequency of the field potential and individual electrical stimulation coincided at stimulus frequencies≤5 Hz, but did not fully coincide at those ≥10 Hz except for the first potential, and that there was no change in the mean arterial blood pressure during stimulation.

Fig. 3a). The response curve started to decline even when the field potentials continued to be recorded (Figs. 2 and 3a; 0.5, 5 and 10 Hz), that is, the peak-time was noted before the cessation of neuronal activity. The peak-time, termination-time and magnitude increased with the stimulus frequency in the range of 0.2–5 Hz, although the values of all three parameters decreased at frequencies≥10 Hz (Figs. 2 and 3).

In most rats, spontaneous oscillations were observed, but could be averaged out by accumulation at 20 data points at stimulus frequencies of 0.5, 5 and 10 Hz. However, the majority of these oscillations still remained after the stimulation period at stimulus frequencies of 0.2 and 50 Hz (Fig. 2) (i.e., the shortest neuronal activity).

**Table 1. Frequency of field potentials for various stimulus frequencies.**

| Stimulus frequency (Hz) | Mean number of field potentials detected |
|---|---|
| 0.2 | 1.0±0.0 |
| 0.5 | 3.0±0.0 |
| 5 | 20.0±4.8 |
| 10 | 8.8±10.4 |
| 50 | 1.0±0.0 |

Mean±SD ($n$=6).

## Time-course of change in RBC velocity and RBC concentration

An important objective of this study was to investigate the time-course of changes in RBC velocity and RBC concentration independently (study 2). We measured the time-courses of changes for these parameters during the application of electrical pulses of 5 s in duration at 5 Hz, because the response curves of RBC velocity and RBC concentration showed poor signal-to-noise ratio at all except this frequency.

There were no significant differences in rise-time and termination-time among LCBF, RBC velocity and RBC concentration (Fig. 4). On the other hand, the peak-time of RBC concentration occurred earlier than those of RBC velocity and LCBF ($p<0.05$, Fig. 4). The peak-times of LCBF, RBC velocity and RBC concentration were 3.2±0.3, 3.5±0.5 and 3.0±0.3 s, respectively ($n$=14).

## DISCUSSION

We have studied the relationship between the field potential recorded and changes in LCBF in response to electrical stimulation of the rat hind paw. Changes in LCBF were detected by LDF, and the time-courses of RBC velocity and RBC concentration were evaluated independently. We showed that even a single pulse of electrical stimulation of the hind paw evoked an increase in LCBF.

**Attributes of LDF.** Before discussing the physiological interpretation of our data, we must know the attributes of LDF. LDF reflects only RBC behavior, and LCBF is calculated as a product of RBC concen-

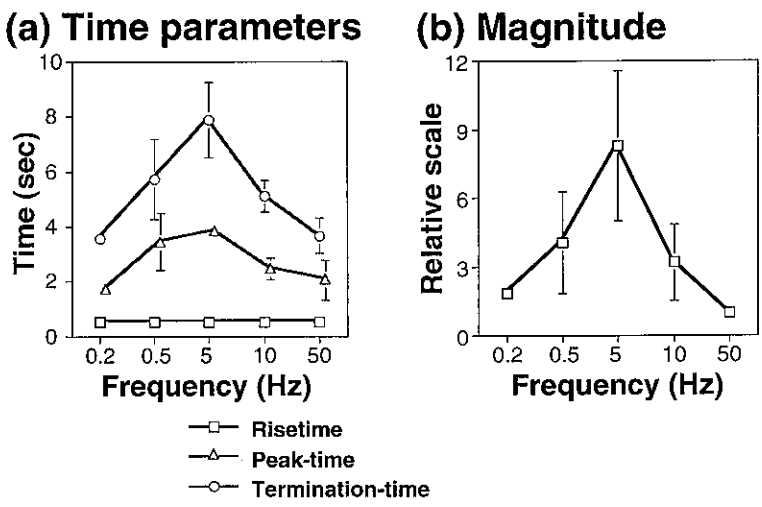


**Fig. 3. Effect of various frequencies of 5-s stimulation on the time parameters and relative response magnitude of LCBF.** (**a**) Changes in rise-time, peak-time and termination-time with the onset of stimulation (see Fig. 1). (**b**) Relative magnitude (area under the LCBF response curve) of the increase in LCBF, with the response magnitude obtained at a stimulus frequency of 50 Hz being considered as 1.0. Note that LCBF rose about 0.5 s after the onset of stimulation regardless of the stimulus frequency (NS), and that LCBF response showed maximal increase at the stimulus frequency of 5 Hz ($p<0.01$). Error bars are ±1 standard deviation.

tration and RBC velocity. True LCBF is a function of both RBC flow and plasma flow. It is known that 10–20% of the capillaries are perfused only by plasma [12], which does not contribute to LDF signals. Therefore, our data may not represent true LCBF if the hematocrit were to vary. In the microvasculature, RBCs flow at the center of the vessel at a speed greater than the plasma velocity (Fahraeus effect [13]), which results in a reduced microvascular hematocrit. In the capillary bed, on the contrary, hematocrit is known to increase slightly by the pathway effect [14]. It is also known that, at microvascular bifurcations, RBC streams into a vessel of which the flow velocity is faster (network Fahraeus effect [13, 14]). These phenomena are probably influenced by the arteriole and/or capillary changes during cortical activation. However, it was found that the local hematocrit did not change [15], or decreased only slightly with an increase in LCBF [16]. Moreover, Dirnagl *et al.* demonstrated that relative CBF measured by LDF correlated well with that measured by [$^{14}$C] iodoantipyrine autoradiography [17], suggesting that relative LCBF as detected by LDF reflects the true LCBF.

**Relationship between neuronal activation and increase in LCBF.** The intensity of cortical activation is evaluated by the amplitude of the evoked potential using a surface electrode. In the cat somatosensory cortex, the maximum hyperemia and maximum amplitude of the evoked potential were both obtained for stimulation at 2–3 Hz [8]. On the other hand, Ibáñez *et al.* [7] reported that regional CBF showed a maximum increase following median nerve stimulation at 4 Hz, despite a relative reduction in the amplitude of the somatosensory evoked potential. Such surface-evoked potentials are difficult to evaluate from the electrophysiological point of view because of the summation of both temporal and spatial electrical activities in a large area. The changes in amplitude reflect a sum of the excitatory and inhibitory activities. Therefore, the amplitudes of surface-evoked potentials do not directly reflect the intensity of cortical activation.

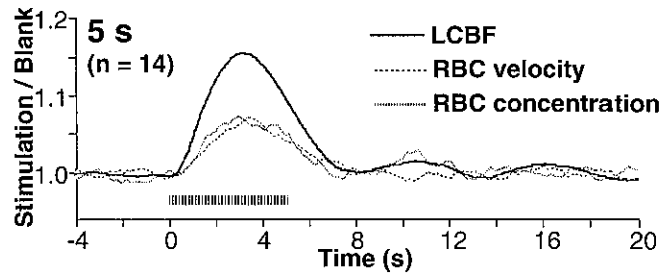


**Fig. 4. Time-course of change in LCBF (solid line), RBC velocity (rough dotted line) and RBC concentration (close dotted line) in response to 5-s stimulations at a frequency of 5 Hz to the hind paw.** Data of the change in LCBF, RBC velocity and RBC concentration were averaged by the number of animals used. Hatched lines indicate stimulus period. Note that RBC concentration peaked before RBC velocity and LCBF ($p<0.05$), and that there was no significant time lag between the rise-times of RBC velocity and RBC concentration.

In this study, field potential was measured through an electrode inserted into the cortex. The frequencies of the field potentials and individual electrical pulses coincided for stimulation at frequencies≤5 Hz, but not for that at frequencies≥10 Hz in most rats (Fig. 2). This implies that the number of field potentials recorded in response to stimulation at frequencies≥10 Hz was much smaller than that recorded at a stimulus frequency of 5 Hz, or that the field potentials recorded after the second pulses were markedly decreased in amplitude at stimulus frequencies≥10 Hz (Fig. 2, Table 1). It is unlikely that cortical neurons have a reaction limit for stimulation at ≥10 Hz because individual somatosensory neurons in the rat and cat cortex

react to stimuli≥10 Hz [18, 19]. While it is possible that such reductions in somatosensory neuronal activation are due to either inhibitory mechanisms, habituation of the peripheral nerve of the hind paw, or limitations of transmission of the sensory input, we considered that it was most likely to be due to inhibitory mechanisms [7]. Indeed, intracortical inhibition was confirmed as a frequency-dependent response above 20 Hz [18], suggesting that inhibitory neurons in the cortex are activated at higher frequencies. These observations suggest that maximum cortical activation by stimulation of the somatosensory area of the hind paw occurred at a stimulus frequency of 5 Hz in our study.

LCBF also showed the maximum increase at a stimulus frequency of 5 Hz ($p<0.01$). At stimulus frequencies≥10 Hz, the increase in LCBF was attenuated with increasing stimulus frequency (Figs. 2 and 3b). The relationship between stimulus frequency and increase in LCBF was previously investigated in the rat somatosensory cortex. Ngai and colleagues demonstrated that the change in diameter of the pial arteriole was maximal in response to sciatic nerve stimulation at 5 Hz [20], and the LCBF showed a similar response profile with pial arteriole change [1]. In the rat whisker barrel cortex, the peak amplitude of blood flow evoked with stimulation at a frequency of 3 Hz was significantly greater than that at other frequencies [21]. These results suggest that the LCBF in the somatosensory area is maximal when the peripheral nerves are stimulated at a frequency of approximately 5 Hz.

The oxygen supply to the tissue may be increased during cortical activation. This phenomenon is evident from the results of optical imaging; that is, an increase in deoxyhemoglobin concentration occurs at the early period of cortical activation [2]. In the present study, we showed that LCBF increased in a similar manner to the intensity of cortical activation, indicating the coupling between cortical activation and increase in LCBF. On the other hand, Fox and Raichle found a quantitative uncoupling of LCBF and oxidative metabolism [22]. These suggest that the increase in LCBF depends on cortical activation, but oxygen consumption is independent from cortical activation. However, the elucidation of this odd phenomena (i.e., the uncoupling between an increase in LCBF and oxygen consumption) needs further investigation, such as the simultaneous measurement of LCBF and direct tissue $P_{O_2}$ with high temporal resolution during cortical activation.

Although spontaneous oscillations could be averaged out by the accumulation of data at stimulus frequencies of 0.5, 5 and 10 Hz, a considerable proportion still remained at those of 0.2 and 50 Hz (Fig. 2). Morita *et al.* [23] reported that pronounced vasodilatation led to the total abolition of vasomotion. This suggests the possibility that spontaneous oscillations are decreased by large increases in LCBF. On the other hand, Golanov *et al.* [24] proposed a neurogenic origin for at least one kind of low-frequency oscillation in the cortex. Our results suggested that spontaneous oscillations were synchronous with stimulation when the strength of cortical activation was small. This was supported by the relatively lower amplitude of the accumulation curve during the pre-stimulus period as compared to that following stimulation. These findings confirm that neural activity is probably related to the oscillations, but the source of these oscillations still remains controversial [25].

**Rise-time of change in LCBF.** In order to discuss the time-course of change in LCBF, it is necessary to confirm that the LDF probe was located at the cortical activation area of the somatosensory cortex for the hind paw. The rise-time of LCBF may be influenced by the location of the LDF probe. In all of the rats in our experiments, the microelectrode was inserted into the cortex at the same location as the LDF probe, and the field potentials were recorded. The mean latency of the field potentials was 10.1±0.7 ms. This agreed with the latency reported in other literature [26]. This indicates that the LDF probe was located at the activation area of the somatosensory cortex of the hind paw.

The LCBF response, for all of the stimulation frequencies examined, rose rapidly about 0.5 s after the onset of stimulation (Fig. 3a). Several investigators have reported a much later rise-time of the LCBF response to somatosensory stimulation. Schmitz *et al.* described that the CBF typically increased within 2–3 s after the onset of forepaw stimulation [6]. A similar time-course for the increase in CBF following cortical stimulation has been observed using modified hydrogen clearance [8]. However, the accuracy of these results was limited either by low signal-to-noise (*S/N*) ratio or poor time resolution. We improved the *S/N* ratios by accumulating 20 to 50 events of repeated trials and time resolution by using a small time constant (0.03 s) in LDF. Therefore, the rise-time of LCBF of 0.5 s was considered to be more reliable compared to previous reports [6, 8].

The physiological interpretation of this 0.5 s response, with respect to capillary and arteriole functions, is still open to debate. The mechanisms of coupling between neuronal activation and LCBF are still under investigation. Several possible mechanisms

have been proposed [27, 28]; for example, reaction caused by metabolic by-products, regulation by cations or chemical substrates [3, 6, 29–31], and neurogenic regulation [32]. In addition, a direct reaction on the capillary has been proposed [12, 27, 33, 34]. Since our method only provides information based on RBC motion within the capillary, further investigations are required for elucidation of the regulatory mechanisms.

**Possible microcirculatory regulation.** In this study, we demonstrated the independent dynamics of RBC velocity and RBC concentration using LDF. The increase in RBC velocity at the capillary level is supposed as being induced by an increase in transluminal pressure due to a decrease in resistance of the upstream arteriole. On the other hand, the increase in RBC concentration within the capillary suggests a direct change in the capillary (i.e., physiological recruitment [35] or capillary dilatation [12, 33]).

Some possible mechanisms of LCBF regulation are proposed. The first is that active capillary changes occur earlier than those in the arteriole [2, 36]. If this were the case, the RBC concentration would increase earlier than the RBC velocity. Actually, there is a report that the capillaries contract under hyperoxia and dilate under hypoxia [34]. In addition, the caliber of the capillary, previously considered to be unchangeable [37], was reported to be altered by carbon dioxide [12, 33]. The second possibility is that the changes in LCBF are primarily controlled only by resistance vessels (i.e., arteriole [38, 39], and passive capillary changes may also be involved. If this were the case, the RBC velocity in the capillary bed would change earlier than the RBC concentration. However, we found no significant time differences between the rise-times of RBC velocity and RBC concentration during somatosensory stimulation, and also found that both RBC velocity and RBC concentration contributed equally to the observed changes in LCBF (Fig. 4). Therefore, we propose a third possibility that both capillary and arteriole changes occur simultaneously and contribute equally to the initial increase in LCBF.

We showed that the RBC concentration peaked before LCBF and RBC velocity ($p<0.05$) (Fig. 4). This earlier peaking of RBC concentration suggests the occurrence of active capillary changes because, if only arteriole changes were involved in LCBF regulation, the changes in RBC concentration would follow those of RBC velocity and LCBF. In summary, it is possible that the capillary and arteriole are controlled by independent mechanisms, and that LCBF is regulated by both capillary and arteriole changes, although arteriolar dilatation, physiological recruitment and capillary dilatation would have to interact in a highly complex manner to regulate LCBF.

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## REFERENCES

1. Ngai AC, Meno JR, and Winn HR: Simultaneous measurements of pial arteriolar diameter and laser-Doppler flow during somatosensory stimulation. J Cereb Blood Flow Metab 15: 124–127, 1995
2. Malonek D and Grinvald A: Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. Science 272: 551–554, 1996
3. Akgören N, Dalgaard P, and Lauritzen M: Cerebral blood flow increases evoked by electrical stimulation of rat cerebellar cortex: relation to excitatory synaptic activity and nitric oxide synthesis. Brain Res 710: 204–214, 1996
4. Barfod C, Akgören N, Fabricius, M, Dirnagl U, and Lauritzen M: Laser-Doppler measurements of concentration and velocity of moving blood cells in rat cerebral circulation. Acta Physiol Scand 160: 123–132, 1997
5. Lindauer U, Villringer A, and Dirnagl U: Characterization of CBF response to somatosensory stimulation: model and influence of anesthetics. Am J Physiol 264: H1223–H1228, 1993
6. Schmitz B, Böttiger BW, and Hossmann KA: Brief hypercapnia enhances somatosensory activation of blood flow in rat. J Cereb Blood Flow Metab 16: 1307–1311, 1996
7. Ibáñez V, Deiber MP, Sadato N, Toro C, Grissom J, Woods RP, Mazziotta JC, and Hallett M: Effects of stimulus rate on regional cerebral blood flow after median nerve stimulation. Brain 118: 1339–1351, 1995
8. Leniger-Follert E and Hossmann KA: Simultaneous measurements of microflow and evoked potentials in the somatosensory cortex of the cat brain during specific sensory activation. Pflügers Arch 380: 85–89, 1979
9. Nilsson GE: Signal processor for laser Doppler tissue flowmeters. Med Biol Eng Comput 22: 343–348, 1984
10. Fukuda O, Endo S, Kuwayama N, Harada J, and Takaku A: The characteristics of laser-Doppler flowmetry for the measurement of regional cerebral blood flow. Neurosurgery 36: 358–364, 1995
11. Nilsson GE, Tenland T and Öberg PÅ: Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. IEEE Trans Biomed Eng BME-27: 597–604, 1980
12. Villringer A, Them A, Lindauer U, Einhäupl K, and Dirnagl U: Capillary perfusion of the rat brain cortex. An in vivo confocal microscopy study. Circ Res 75: 55–62, 1994
13. Pries AR, Ley K, and Gaehtgens P: Generalization of the Fahraeus principle for microvessel networks. Am J

Physiol 251: H1324–H1332, 1986
14. Pries AR, Secomb TW, and Gaehtgens P: Relationship between structural and hemodynamic heterogeneity in microvascular networks. Am J Physiol 270: H545–H553, 1996
15. Chen JL, Wei L, Acuff A, Bereczki D, Hans FJ, Otsuka T, Finnegan W, Patlak C, and Fenstermacher L: Slightly altered permeability-surface area products imply some cerebral capillary recruitment during hypercapnia. Microvasc Res 48: 190–211, 1994
16. Todd MM, Weeks JB, and Warner DS: Cerebral blood flow, blood volume, and brain tissue hematocrit during isovolemic hemodilution with hetastarch in rats. Am J Physiol 263: H75–H82, 1992
17. Dirnagl U, Kaplan B, Jacewicz M, and Pulsinelli W: Continuous measurement of cerebral cortical blood flow by laser-Doppler flowmetry in a rat stroke model. J Cereb Blood Flow Metab 9: 589–596, 1989
18. Hellweg FC, Schultz W, and Creutzfeldt OD: Extracellular and intracellular recording from cat's cortical whisker projection area: thalamocortical response transformation. J Neurophysiol 40: 463–479, 1977
19. Simons DJ: Response properties of vibrissa units in rat SI somatosensory Neocortex. J Neurophysiol 41: 798–820, 1978
20. Ngai AC, Ko KR, Morii S, and Winn HR: Effect of sciatic nerve stimulation on pial arterioles in rats. Am J Physiol 254: H133–H139, 1988
21. Woolsey TA, Rovainen CM, Cox SB, Henegar MH, Liang GE, Liu D, Moskalenko YE, Sui J, and Wei L: Neuronal units linked to microvascular modules in cerebral cortex: response elements for imaging the brain. Cereb Cortex 6: 647–660, 1996
22. Fox PT and Raichle ME: Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc Natl Acad Sci USA 83: 1140–1144, 1986
23. Morita Y, Hardebo JE, and Bouskela E: Influence of cerebrovascular sympathetic, parasympathetic, and sensory nerves on autoregulation and spontaneous vasomotion. Acta Physiol Scand 154: 121–130, 1995
24. Golanov EV, Yamamoto S, and Reis DJ: Spontaneous waves of cerebral blood flow associated with a pattern of electrocortical activity. Am J Physiol 266: R204–R214, 1994
25. Mayhew JEW, Askew S, Zheng Y, Porrill J, Westby GWM, Redgrave P, Rector DM, and Harper RM: Cerebral vasomotion: a 0.1-Hz oscillation in reflected light imaging of neuronal activity. Neuroimage 4: 183–193, 1996
26. Claus D and Neundörfer B: Remarks peripheral and central neural conduction in the rats. Z EEG-EMG 14: 160–163, 1983
27. Villringer A and Dirnagl U: Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. Cerebrovasc Brain Metab Rev 7: 240–276, 1995
28. Lou HC, Edvinsson L, and MacKenzie ET: The concept of coupling blood flow to brain function: revision required? Ann Neurol 22: 289–297, 1987
29. Dirnagl U, Lindauer U, and Villringer A: Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. Neurosci Lett 149: 43–46, 1993
30. Ko KR, Ngai AC, and Winn HR: Role of adenosine in regulation of regional cerebral blood flow in sensory cortex. Am J Physiol 259: H1703–H1708, 1990
31. Urbanics R, Leniger-Follert E and Lübbers DW: Time course of changes of extracellular $H^+$ and $K^+$ activities during and after direct electrical stimulation of the brain cortex. Pflügers Arch 378: 47–53, 1978
32. Sato A and Sato Y: Regulation of regional cerebral blood flow by cholinergic fibers originating in the basal forebrain. Neurosci Res 14: 242–274, 1992
33. Duelli R and Kuschinsky W: Changes in brain capillary diameter during hypocapnia and hypercapnia. J Cereb Blood Flow Metab 13: 1025–1028, 1993
34. Tomita M, Fukuuchi Y, Tanahashi N, Kobari M, Takeda H, Yokoyama M, Ito D, and Terakawa S: Contraction/dilatation of cultured vascular endothelial cells inducted by hyperoxia/hypoxia. J Cereb Blood Flow Metab 15 (Suppl): S271, 1995
35. Kuschinsky W and Paulson OB: Capillary circulation in the brain. Cerebrovasc Brain Metab Rev 4: 261–286, 1992
36. Malonek D, Dirnagl U, Lindauer U, Yamada K, Kanno I, and Grinvald A: Vascular imprints of neuronal activity: relationships between the dynamics of cortical blood flow, oxygenation, and volume changes following sensory stimulation. Proc Natl Acad Sci USA 94: 14826–14831, 1997
37. Mchedlishvili G and Varazashvili M: Hematocrit in cerebral capillaries and veins under control and ischemic condition. J Cereb Blood Flow Metab 7: 739–744, 1987
38. Paulson OB and Newman EA: Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? Science 237: 896–898, 1987
39. Segal SS and Duling BR: Flow control among microvessels coordinated by intercellular conduction. Science 234: 868–870, 1986