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Evaluation of Absolute Cerebral Blood Flow by Laser-Doppler Scanning – Comparison with Hydrogen Clearance

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Key Words
Cerebral blood flow · Laser-Doppler flowmetry · Laser-Doppler scanning · Global ischemia · Rat

Abstract
A major limitation of laser-Doppler (LD) flowmetry, which enables noninvasive and continuous recording of tissue perfusion, is its inability to evaluate the absolute cerebral blood flow (CBF). Using a computer-controlled micromanipulator, the LD scanning technique provides information on the brain microcirculation in many different locations, information which is not available from a single stationary probe. The purpose of the current study was to examine whether LD scanning estimates can be calibrated for the absolute CBF by comparing LD scanning with the hydrogen clearance (HC) method. In Wistar rats (n = 31) including old rats (122–123 weeks old, n = 8), the CBF was altered using the global ischemia model by bilateral carotid artery occlusion coupled with hypobaric hypotension. The CBF was determined simultaneously by the LD scanning technique and HC at each mean arterial blood pressure step, and the correlation of CBF between the two techniques was analyzed. CBF measured by LD scanning was expressed as LD units. Absolute CBF values obtained by methods were correlated (r = 0.87), and the formula to calibrate absolute CBF values from LD units was y = 1.8x – 0.6. On the other hand, in old rats the formula to calibrate the absolute values was different (y = 1.3x + 8.3, r = 0.85). The results suggest that CBF data obtained by LD scanning could be calibrated into absolute blood flow values in particular circumstances, and that LD scanning could compensate in part for the weakness of LD flowmetry.

Although various methodologies for measuring cerebral blood flow (CBF) have been developed, all present advantages and disadvantages. Therefore, an ideal method for continuous and noninvasive monitoring with high spatial resolution has not yet been established.

Laser-Doppler (LD) flowmetry has been a practical method for measuring blood flow continuously in a noninvasive manner and is no longer considered just a research tool but is also used in clinical investigations [1]. Blood flow is calculated from the Doppler shift of the low-power laser light reflected by moving red blood cells, and the volume measured by LD flowmetry is usually about 1–2 mm^3, which provides local CBF (lCBF) rather than regional CBF (rCBF). However, the values of blood flow are not expressed as absolute flow values (ml/g/min) but in LD units relative to the initial values.

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The changes in blood flow detected by LD flowmetry have been compared with other measurements performed by the hydrogen clearance (HC) method [2, 3], microspheres [4] and autoradiography [5]. These reports suggested that LD flowmetry does not provide accurate measurements of absolute lCBF values, but it facilitates accurate measurement of changes in lCBF. However, it is important to use absolute values to compare individual subjects or a critical state such as ischemia in clinical or experimental studies. Recently, an LD scanning technique has been reported [6–10]. This method is superior to conventional LD flowmetry in terms of spatial resolution, i.e. from lCBF to rCBF [7].

Therefore, we studied the changes in CBF in global ischemia using the LD scanning technique and HC method simultaneously and evaluated the correlation between the CBF values obtained from the two systems. The goal of the present study was to examine whether it is possible to calibrate the CBF detected by LD scanning into absolute blood flow values.

Materials and Methods

This experiment was conducted in accordance with the recommendations on the establishment of animal experiment guidelines approved at the 80th General Assembly of the Science Council (1980) and the Experimental Animal Society (1987) of Japan.

Animal Preparation

We divided the animals into two groups: young and old animals. Twenty-three male Wistar rats (8–11 weeks) weighing 310–380 g and 8 old (122–123 weeks) Wistar rats were premedicated with 0.5 mg atropine, and anesthetized with chloral hydrate (36 mg/100 g i.p.). Anesthesia was maintained by chloral hydrate (12 mg/100 g i.p.) given hourly through a peritoneal catheter. Then endotracheal intubation was performed, and rats were ventilated artificially with a mixture of 70% nitrous oxide and 30% oxygen after muscle relaxation with pancuronium (0.6 mg/kg). Temporal muscle and rectal temperatures were controlled at 37°C using a feedback-controlled heating blanket (CMA 150, Carnegie Medicine, Stockholm, Sweden) and lamp (IFR 100, Unique Medical, Tokyo, Japan). Both carotid arteries were exposed and isolated carefully from the surrounding tissue, using an operating microscope (OP microscope; Zeiss, Wetzlar, Germany). A 3-0 silk thread was looped around the right carotid artery to occlude the artery by attaching a 15-gram weight to the end of the thread. The left carotid artery was cannulated with polyethylene catheters to enable the continuous monitoring of mean arterial blood pressure (MABP; Polygraph system RM-600, Nihon Koden, Tokyo, Japan) and to provide serial measurements of PaO_2, $PaCO_2$ and pH (ABL3 blood gas analyzer). The animal's head was placed in a stereotactic frame (SR-6, Narishige, Japan). After a 15-mm midline skin incision, a craniotomy (4 × 5 mm) was made over the frontoparietal region using a high-speed drill under the operating microscope. During the craniotomy, great care was taken not to damage the brain surface.

rCBF Measurement by LD Scanning

rCBF was measured by LD flowmetry (ALF-21, Advance, Tokyo, Japan) using a 0.8-mm needle probe. The LD flowmetry provides lCBF information with a stable and low biological zero (<1.0 LD units). The rCBF was expressed in LD units. It was measured at 25 (5 × 5) locations in a scanning procedure using a motor-driven and computer-controlled micromanipulator. There was random registration of the results from 25 individual measurements at one scanning location and information obtained from 25 different locations at 500 μm intervals. To avoid artifacts caused by measurements recorded when the probe was still moving, a delay of 2 s was allowed before each measurement. The mean value calculated from 20 samples at a single location was used as the lCBF value in each place with a sample interval of 100 ms; one scan took approximately 2 min. The technique permits repeated scans from a given set of locations.

Hydrogen Clearance

For HC recordings we used a epoxylite insulated platinum electrode with 0.1 mm tip diameter, exposed 1.0 mm. The platinum electrode was inserted into the right parietal cortex 1 mm in depth adjacent to the region, where the needle probe of LD flowmetry was placed, without damaging any cerebral vessels. As a reference, a standard calomel electrode was placed subcutaneously in the dorsal neck region. Hydrogen gas (a concentration of 3–5%) was given through the tracheal tube until the hydrogen current was sufficient for a steady state, and then the inhalation of hydrogen gas was stopped. The recordings of exponential washouts were taken over 3 min. The CBF values were obtained by monoexponential analysis of the hydrogen washout using an UH meter (MHG-D, Unique Medical, Tokyo, Japan).

Bilateral Global Brain Ischemia by Hypobaric Hypotension

The lower portion of the body was placed in a sealable chamber, connected to an electronically controlled vacuum pump for the induction of the hypobaric hypotension method. The barometric pressure within the chamber could be reduced, and hypotension was caused by the pooling of venous blood in the lower half of the body.

Experimental Protocol

CBF was measured by LD scanning and HC for control data. Next the right carotid artery was occluded by pulling the 3-0 silk thread which was looped around the artery. After 5 min, the MABP was reduced by hypobaric hypotension to 70 mm Hg and in 5 mm Hg steps down to 40 mm Hg. The MABP was immediately reduced to the intended level and was then maintained constant for 10 min. During this plateau phase, MABP was measured continuously, and lCBF was also recorded at the 25 locations by LD scanning and HC simultaneously in the right parietal cortex. LD scanning measurement was started at the same time as the HC measurement, which began after the animal had inhaled sufficient H_2 gas.

In addition, another 5 rats (age 8–11 weeks) received only a craniotomy as sham-operated controls.

Statistical Analysis

Data are expressed as means ± SEM or as the mean percentage of baseline values ± SEM. Differences in the physiological variables were evaluated by analysis of variance (ANOVA). Statistical significance was accepted at an error probability of $p < 0.05$. All statistics were done by Sigma-Stat software (Jandel Scientific, Erkrath, Germany).

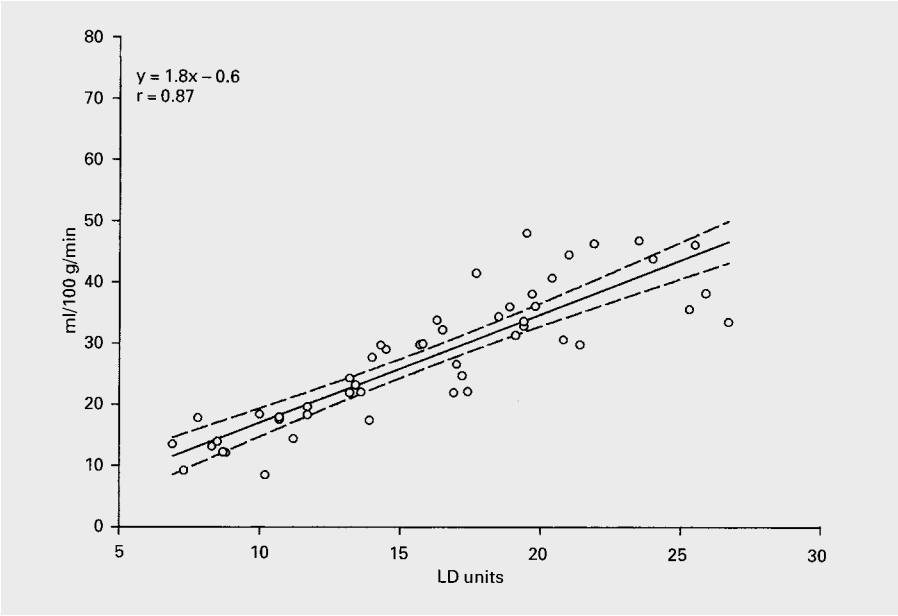

Fig. 1. Correlation between the absolute values of CBF measured by LD scanning (LD units) and HC (ml/100 g/min) in young rats. Points and dotted lines represent the CBF and 95% confidence interval of the results, respectively. CBF values obtained from LD scanning correlated well with the absolute values of CBF obtained by HC ($y = 1.8x - 0.6$, $r = 0.87$; $p < 0.001$, $n = 50$).

Table 1. CBF changes during hypotension in young and old rats (means ± SEM)

Rats		Blood pressure						
		70 mm Hg	65 mm Hg	60 mm Hg	55 mm Hg	50 mm Hg	45 mm Hg	40 mm Hg
Young	LD scanning, LD units	24.4±7.6	24.0±4.0	21.1±3.6	19.4±3.3	17.0±3.2	13.2±2.4	10.3±1.6
	HC, ml/100 g/min	50.0±10.2	43.5±5.1	38.0±4.4	31.5±3.5	24.9±3.1	20.8±2.7	14.1±2.1
Old	LD scanning, LD units	22.6±2.2	20.9±2.3	18.7±1.3	14.1±2.6	14.3±1.2	12.2±1.3	9.9±0.7
	HC, ml/100 g/min	44.0±2.5	37.0±5.3	30.8±5.1	26.0±5.4	24.0±3.7	22.7±3.4	20.0±1.9

Results

Physiological Variables

The arterial pO_2, pCO_2 and pH in the initial nonhypotensive state and in hypotensive (MABP = 40 mm Hg) state remained within the normal range, and there were no significant differences in the physiological parameters of each group between normal and hypotensive states (data not shown).

Control CBF

CBF values obtained by LD scanning in the sham-operated group were expressed in LD units. The initial mean CBF by LD scanning was 27.1 ± 4.0 LD units (physiological rCBF), and this value did not change significantly throughout the experiments (data not shown). In HC, the CBF in the right parietal cortex was 60.4 ± 10.3 ml/100 g/min, which, similar to LD scanning, did not change.

CBF Changes during Hypotension

In the young and old rats, the initial (physiological) rCBF and lCBF values were 29.9 ± 2.8 LD units and 66.3 ± 8.8 ml/100 g/min, 28.1 ± 4.5 LD units and 53.6 ± 6.2 ml/100 g/min, respectively. Table 1 shows the rCBF measured by LD scanning and the lCBF measured by HC when MABP was reduced from 70 to 40 mm Hg in intervals of 5 mm Hg. The CBF measured by both methods decreased in relation to the reduction in MABP.

Comparison of CBF Measured by Two Different Methods

We compared the results of CBF measurements obtained by LD scanning and HC for all MABP steps from normal to 40 mm Hg of MABP. Figure 1 indicates that there was a correlation between the rCBF measured by LD scanning and rCBF measured by HC in the young

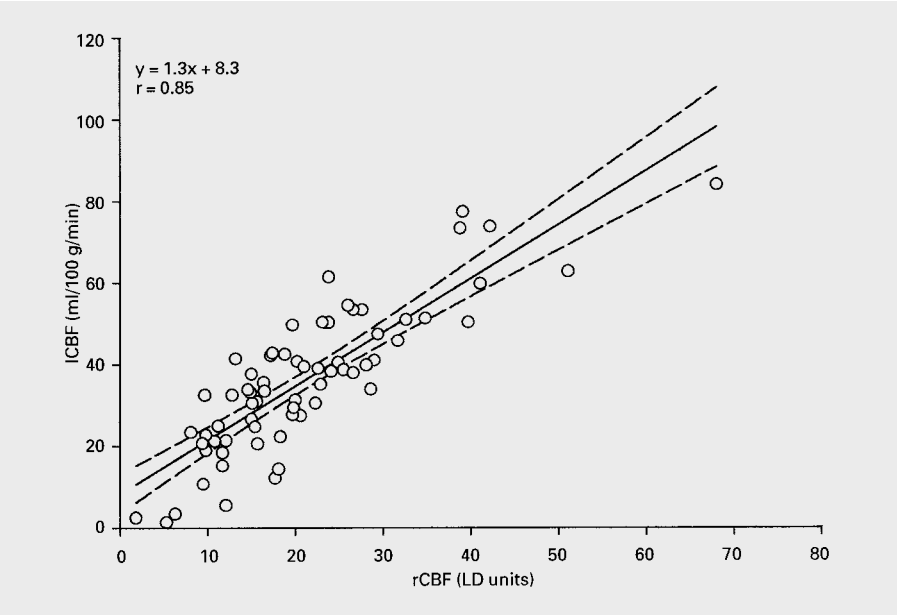

Fig. 2. Correlation of the absolute values of CBF measured by LD scanning (LD units) and HC (ml/100 g/min) in old rats. This correlation is also less than that in young rats. Additionally, the formula differs between young and old rats ($y = 1.3x + 8.3$; $p < 0.001$, $n = 69$).

group. It demonstrated a strong correlation, with a correlation coefficient (r) of 0.87.

The formula to calibrate LD units into absolute blood flow values (ml/100 g/min) was: $y = 1.8x - 0.6$ (x: LD units, y: ml/100 g/min). In the old group, the correlation between the rCBF measured by LD scanning and HC was slightly lower than in the young group ($r = 0.85$, fig. 2). The formula to calibrate LD units into absolute blood flow values was $y = 1.3x + 8.3$, which differed from that of the young group.

Discussion

LD Flowmetry

Although many methods for measuring CBF, e.g. LD flowmetry, HC, [^{14}C]iodoantipyrine (IAP) and microspheres, have been developed, each method has both strengths and weaknesses. HC, IAP, and microspheres provide absolute blood flow values. However, microspheres and IAP can only offer discontinuous measurements and furthermore, for IAP the animals must be sacrificed. HC provides repeated measurements with reasonable quantitative estimates, but only on an intermittent basis and in addition injury to the tissue occurs. LD flowmetry is relatively noninvasive and allows for rapid and continuous measures of blood flow. However, LD flowmetry has an ill-defined spatial resolution (the spatial resolution is limited to a volume of 1–2 mm^3), and therefore is of limited use in the evaluation of absolute CBF. It is also highly dependent on the localization of the LD probe and the underlying anatomical substrate.

Comparison of LD Flowmetry and Other Methods

To date, many investigators have demonstrated a good correlation in the percent changes in blood flow values between LD flowmetry and other methods such as HC [2, 3, 11–13], the microsphere technique [14] and [^{14}C]IAP [15]. Skarphedinsson et al. [3] reported that the CBF in the parietal cortex using HC was 158.6 ± 11.5 (fast CBF), 29.1 ± 1.6 (slow CBF) and 83.3 ± 7.4 (mean CBF). They used a biexponential curve to calculate the CBF. They also showed a linear relationship between relative values of changes in blood flow (percent of control) obtained with the HC method and LD flowmetry. In their report the coefficients were 0.658, 0.876 and 0.878 for the correlation between the LD flowmetry and HC data for relative changes in fast, slow and weighted mean CBF, respectively. Rundquist et al. [16] demonstrated a linear relationship between the blood flow of the sciatic nerve in rats and the LD flowmetry signal (in volts) by comparing the signal with the flow measured by [^{14}C]IAP infusion. They reported that the LD flowmetry signal from the sciatic nerve of the rat was linearly related to nerve blood flow measured by [^{14}C]IAP, with a coefficient of 0.73. Haberl et al. [13] examined a possible correlation between LD flowmetry and HC using a cat model, where CBF was changed by hypocapnia or norepinephrine-induced hypertension.

They demonstrated a good correlation between the percent changes in blood flow measured by LD flowmetry and HC. They showed that the slope of the line relating HC and LD flowmetry was 0.94 and the correlation coefficient was 0.97. Arbit et al. [17] compared CBF in rats measured by LD flowmetry or HC using a combination of LD flowmetry and the HC probe. They observed an excellent linear correlation (r = 0.988) and the calibration factor was 8 ml/100 g/min/kHz.

LD Scanning

In the current study, we used the LD scanning technique for measuring the CBF in the ischemic and reperfused rat brain. LD scanning is a method suited for studies where lCBF inhomogeneities are expected, as it can better assess the rCBF than conventional LD flowmetry. This technique allows for the detection of the rCBF from lCBF data by analyzing blood flow values at many points of an experimental animal's cortex. The advantage of this method is the high spatial resolution proposed [7–10, 18]. In these studies, sample sizes above n = 25 were necessary to obtain more reliable information on rCBF. Therefore, to obtain valid rCBF information and in order to reduce the measurement time as much as possible we selected 25 points. Indeed, it took about 2 min to measure rCBF. Measurements may be even quicker (1 min) than in the present study by changing the sampling interval. To represent CBF, we used the term LD units and not percent changes because the LD scanning technique is capable of detecting more regional values.

Recently, Nakase et al. [8–10] reported on the microcirculatory events following disturbed venous circulation in the rat brain using this scanning technique. They utilized three-dimensional cortical CBF mapping and frequency histograms from the CBF data obtained by scanning, and revealed details on the profound topographical changes in the microcirculation in venous circulatory perturbation.

Changes in CBF following Global Ischemia

We induced global ischemia using the hypobaric hypotension technique. This method can induce any intended blood pressure level without heparinization, bleeding, or the additional occlusion of the vertebral arteries [6]. Accordingly, this model was suitable for evaluating the changes in CBF under a variety of hypotensive states. In the current study, rCBF detected by LD scanning and lCBF found by HC were reduced in proportion to the changes in MABP.

Calibration of LD Units into Absolute Values

The purpose of this study was to detect the absolute values of CBF in a noninvasive manner using the LD scanning technique. It is more suitable to evaluate a variety of conditions with the absolute blood flow values rather than the percent change of CBF, for example if an investigator tries to establish the critical flow thresholds in severely compromised conditions, i.e. ischemic penumbra or infarcted tissue. Additionally, it might be possible to compare data from different experimental subjects. So far, there have been no reports on a calibration to absolute blood flow values from LD flowmetry. Dirnagl et al. [5] reported that absolute rCBF values recorded with LD flowmetry correlated poorly (r = 0.54) with [^{14}C]IAP measurements in contrast to the very good correlation of the relative LD flowmetry readings. They stated that the discrepancy between the CBF with both methods may be explained by several factors. The rat pial microvasculature is very dense, so the probe of LD flowmetry has to be placed in the proximity of a large pial artery or vein, which might superimpose over the true microvascular flow signal with a high flow velocity and volume component. In addition, small movements of the probe can result in major changes in the flow signal. In this study, a comparison between LD units and the absolute CBF values (ml/100 g/min) with HC demonstrated a good correlation especially within a certain range of pathological CBF values. The calibration formula concluded from our study was $y = 1.8x - 0.6$ ($r = 0.87$).

In a comparison of CBF mesured by both methods in old rats, the results differed compared to those in young rats, and the correlation between LD scanning and HC was slightly lower. In old rats, the interindividual difference may be high and the thickness of the cortex is thinner than that of young rats. The HC probe was placed at a depth of 1 mm in our study, so the absolute CBF values detected by HC may be affected by the flow in the white matter in old rats.

Although the correlation between LD units and absolute blood flow values (ml/100 g/min) obtained by HC was high, an important point to remember is that we measured the CBF of the parietal cortex with LD scanning and HC simultaneously. However, the measurements locations were not identical in the two methods. With the LD scanning technique, we measured at 25 locations (covering an area of 2.5×2.5 mm^2) and thus obtained a median rCBF; with HC, the measured area was only 1×1 mm around the probe. Therefore, the LD technique may be advantageous for rCBF measurements. On the other hand, LD can only detect flow to a depth of about

1 mm, while HC can measure 1 mm above and below the probe (2 mm in all). Thus each method has its advantages and disadvantages, although a good correlation was found between the two methods.

In conclusion the LD scanning technique could potentially provide absolute rCBF values in a variety of circumstances in a noninvasive manner, and LD scanning can compensate in part for the weakness of LD flowmetry. On the other hand LD scanning loses high temporal resolution since scanning of each of the 25 locations lasts at least 1 s. However, even with this limitation borne in mind, LD scanning will be an attractive and powerful tool.

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References

1 Haberl RL, Villringer A, Dirnagel U: Applicability of laser-Doppler flowmetry for cerebral blood flow monitoring in neurological intensive care. Acta Neurochir Suppl 1993;59:64–68.

2 Kramer MS, Vinall PE, Katolik LI, Simeone FA: Comparison of cerebral blood flow measured by laser-Doppler flowmetry and hydrogen clearance in cats after cerebral insult and hypervolemic hemodilution. Neurosurgery 1996;38:355–361.

3 Skarphedinsson JO, Harding H, Thoren P: Repeated measurements of cerebral blood flow in rats. Comparisons between the hydrogen clearance method and laser Doppler flowmetry. Acta Physiol Scand 1988;134:133–142.

4 Lindsberg PJ, O'Neill JT, Paakkari IA; Hallenbeck JM, Feurstein G: Validation of laser-Doppler flowmetry in measurement of spinal cord blood flow. Am J Physiol 1989;257:674–680.

5 Dirnagl U, Kaplan B, Jacewicz M, Pulsinelli W: Continuous measurement of cerebral blood flow by laser-Doppler flowmetry in a rat stroke model. J Cereb Blood Flow Metab 1989;9:589–596.

6 Heimann A, Kroppenstedt S, Ulrich P, Kempski OS: Cerebral blood flow autoregulation during hypobaric hypotension assessed by laser Doppler scanning. J Cereb Blood Flow Metab 1994;14:1100–1105.

7 Ungersbock K, Heimann A, Strecker U, Kempski O: Mapping of cortical blood flow by laser-Doppler flowmetry; in Tomita M, Mchedlishvili G, Rosenblum WI, Heiss W-O, Fukuuchi Y (eds): Microcirculatory Stasis in the Brain. Amsterdam, Elsevier Science, 1993, pp 405–413.

8 Nakase H, Heimann A, Kempski O: Cerebral blood flow in a rat cortical vein occlusion model. J Cereb Blood Flow Metab 1996;16:720–728.

9 Nakase H, Heimann A, Kempski O: Alterations of regional cerebral blood flow and tissue oxygen saturation in a rat sinus-vein thrombosis model. Stroke 1996;27:720–727.

10 Nakase H, Kempski O, Heimann A, Takeshima T, Tintera J: Microcirculation after cerebral venous occlusion assessed by laser Doppler scanning. J Neurosurg 1997;87:307–314.

11 Casadevall M, Pnes J, Pique JM, Bosch J, Teres J, Rodes J: Limitations of laser-Doppler and reflectance spectrophotometry in estimating gastric mucosal blood flow. Am J Physiol 1992;263:810–815.

12 Diresta GR, Kiel JW, Riedel GL, Kaplan P, Shephead AP: Hybrid blood flow probe for simultaneous H_2 clearance and laser-Doppler velocimetry. Am J Physiol 1987;253:573–581.

13 Haberl RL, Heizer ML, Ellis EF: Laser-Doppler assessment of brain microcirculation: Effect of local alterations. Am J Physiol 1989;256:1255–1260.

14 Talman WT, Dragon DMN, Heistad DD, Ohta H: Cerebrovascular effects produced by electrical stimulation of fastigial nucleus. Am J Physiol 1991;261:707–713.

15 Fabricius M, Lauritzen M: Laser-Doppler evaluation of rat microcirculation: Comparison with the [^{14}C]-iodoantipyrine method suggests discordance during cerebral blood flow increases. J Cereb Blood Flow Metab 1996;16:156–161.

16 Rundquist I, Smith QR, Michel ME, Ask P, Oberg PA, Rapoport SI: Sciatic nerve blood flow measured by laser Doppler flowmetry and [^{14}C] iodoantipyrine. Am J Physiol 1985;248:311–317.

17 Arbit E, DiResta GR, Bedford RF, Shah NK, Galicich JH: Intraoperative measurement of cerebral and tumor blood flow with laser-Doppler flowmetry. Neurosurgery 1989;24:166–170.

18 Kempski O, Heimann A, Strecker U: On the number of measurements necessary to assess regional cerebral blood flow by local laser Doppler recordings: A simulation study with data from 45 rabbits. Int J Microcirc 1995;15:37–42.