Acute hyperglycemia compromises cerebral blood flow following cortical spreading depression in rats monitored by laser speckle imaging

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Abstract. Hyperglycemia and cortical spreading depression (CSD) are possible factors that worsen the outcome of ischemic stroke, and it is probable that there is a longterm cooperative effect of hyperglycemia and CSD on cerebral blood flow (CBF). Long-lasting and full-field observation of changes in CBF following CSD *in vivo* during acute hyperglycemia in rats might show whether this is the case. Here, we utilized laser speckle imaging to study influences of acute hyperglycemia on CBF at the level of individual vascular compartments for 3 h in normal rats and those with CSD. It is shown that there are extensive increases of CBF at the arteriole and parenchyma over the normal rat cortex during acute hyperglycemia, whereas there is no significant change in CBF at the venule. We also find that, at all vascular compartments, after the glucose administration there is a stepwise reduction of CBF following CSD, but after saline injection CBF following CSD is close to the baseline. Our results indicate that acute hyperglycemia could aggravate the severity of decrease in CBF following CSD, suggesting possible mechanisms by which hyperglycemia exacerbates cerebral damage after ischemic stroke. [DOI: 10.1117/1.3041710]

Keywords: cerebral blood flow; glucose; laser speckle; optical imaging; rat.

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1 Introduction

Previous studies have demonstrated that there is a decrease in cerebral blood flow (CBF) in animals with acute or chronic hyperglycemia.[1] Hyperglycemia might have a longterm effect on CBF, especially under the condition of cerebral ischemia. Lanier[2] reported that within 24 h of the onset of cerebral ischemia, even mild hyperglycemia is associated with exacerbated cerebral damage and increased morbidity and mortality; Bruno[3] also described patients with nonlacunar stroke, in which high blood-glucose levels on admission were associated with a worse outcome at 3 months. It has been reported that 20–50% of strokes are accompanied with concomitant hyperglycemia regardless of the presence of pre-existing diabetes.[4] This hyperglycemia occurring during experimental and clinical strokes has been associated with increased cerebral damage.[4]

Cortical spreading depression (CSD) is characterized by transitory, spreading neuronal depolarization that propagates like a wave on the cortical surface. Many studies have shown that normal brain tissue invaded by CSD presents reversible changes in CBF, glucose metabolism, transmembrane ion transport and so on.[5–8] There is evidence that CSD plays an important role in ischemic stroke, and it has been commonly accepted that CSD increases the ischemic volume and aggravates ischemic injury.[8] This deterioration of brain tissue resulting from CSD waves may be caused by its reducing further the already decreased blood flow in the penumbral zone of the ischemic cortex.[8]

Hyperglycemia and CSD can worsen the outcome of ischemic stroke and also influence CBF.[1,8] Therefore, it is possible that both of them have a prolonged cooperative effect on CBF in ischemic stroke. Research on the changes in CBF following CSD during acute hyperglycemia for a long period might provide evidence for this possibility.

To shed light on the spatiotemporal changes in CBF for the different vascular compartments in the cortex, we selectively used a technique of laser speckle imaging to provide a directly spatial pattern of changes in blood flow, avoiding the sampling restriction of the previous techniques. Laser speckle imaging,[9] as a means of enabling CBF imaging at high spatiotemporal resolution, has recently attracted extensive attention, and it has been successfully applied to explore relative changes in CBF induced by CSD,[10] cerebral ischemia,[11,12] and sensory stimulation.[13] It has been demonstrated that not only laser speckle imaging and laser Doppler imaging provide equivalent assessments of blood flow,[14] but also the relative measurement of flow changes by laser speckle imaging can be accurately calibrated by the absolute flow imaging provided by spectral-domain Doppler optical coherence tomography.[15]

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Here, we adopt an acute rat hyperglycemic model, in conjunction with laser speckle imaging, to monitor the long-term effect of acute hyperglycemia and the additional effect of CSD during acute hyperglycemia on CBF for different vascular compartments.

2 Materials and Methods

2.1 *Surgical Operation*

Animal care and experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals at Huazhong University of Science and Technology. Rats, fasted except for water for 12–16 h, were used. Intraperitoneal anesthesia was implemented with a mixture of α-chloralose (50 mg/kg) with urethane (600 mg/kg) in adult male Sprague–Dawley rats (200–300 g). The body temperature of the rats was kept constant at 37.0 ± 0.5 °C with a feedback-controlled heating pad. The left femoral artery was cannulated for serial measurement of arterial pressure and blood gases.

Each rat head was placed in a stereotaxic apparatus. A longitudinal incision about 10 mm was made along the head midline. The skull bone overlying the parietal cortex was removed with a high-speed dental drill (Fine Science Tools, USA) under constant saline cooling to form a 6.0×8.0 mm cranial window with intact dura. The cranial window was bathed with normal saline at 37.0 ± 0.5 °C. A burr hole (2 mm diameter) was drilled in the ipsilateral frontal bone to induce CSD by electrical stimulation. The rats were allowed to recover for at least 1 h.

Acute hyperglycemia in rats ($n=10$) was produced by giving a 50% solution of D-glucose in water at 15 mL/kg intraperitoneally. The same volume of normal saline was given by intraperitoneal injection to the control rats ($n=10$).

2.2 *Monitoring Changes of CBF by Laser Speckle Imaging*

The technical details of laser speckle imaging used in this experimental system have been described elsewhere.[11,16] Briefly, a 17 mW He–Ne laser ($\lambda=632.8$ nm, Melles Griot, USA) was used to illuminate the rat cortex. A 12-bit cooled charge-coupled device camera (Pixelfly, 480×640 pixel array, PCO Computer Optics, Germany) attached to a stereomicroscope (SZX12, Olympus, Japan) was employed for image acquisition. The administration of agents (50% D-glucose or saline) was performed immediately after the acquisition of baseline imaging of laser speckle. Laser speckle imaging was repeated at 30 min intervals after the administration of agents. Ninety consecutive raw speckle images were acquired for each measurement (20 ms exposure time). The whole observation period was 180 min. Speckle contrast images were acquired by analyzing the raw speckle images using the method of laser speckle temporal contrast analysis.[9,17] The images of inverse correlation time values (ICT, $1/\tau$, arbitrary units) were calculated from the speckle contrast images. This ICT image was then used as an indicator of the CBF parameter. Relative changes in CBF at each time in a region were calculated as a percentage of the baseline.

2.3 *CSD Induced by Electrical Stimulation*

In five rats of each group, CSD was initiated by intracortical electrical stimulation (5 mA, 1 s duration, 1 pulse). Optical reflectance and direct current potential were simultaneously recorded to characterize CSD waves as mentioned in Ref 12. At the start, just before the injection of glucose or saline (0 min), a laser speckle image was obtained and then the first CSD was immediately induced. CSD waves were again induced immediately after laser speckle imaging at 30, 60, 90, 120, and 150 min after experimental intervention.

2.4 *Data Analysis*

For data analysis, different vascular compartments were manually chosen by selecting subregions of the ICT image. To selectively study the relative changes in CBF at different cortical vascular compartments, we identified arterioles, capillaries, and venules. Identification of arterioles and venules was done by visual inspection (morphology, pulsation, color of vessels, etc.) through a microscope and a camera. The direct observation of surface capillaries was only rarely possible at this magnification. However, as the parenchyma contains mainly capillaries, signals originating from parenchyma regions contained mostly capillary contributions.

Three subregions of the image were selected for each of the three vascular compartments (arteriole, venule and parenchyma). Relative change in CBF at each time in a subregion was expressed as percent change from its baseline. The same process as those reported here was done in each experiment (including five rats). Statistical analysis was then performed on this dataset, consisting of 15 samples for each vascular compartment at each time point. The relative changes in CBF at each of the three vascular compartments were obtained by averaging over 15 samples, yielding three curves for the time courses of relative changes in CBF for the three vascular compartments.

3 Results

All physiological parameters were maintained within normal limits throughout the experiments.

3.1 *The Effect of Acute Hyperglycemia on CBF in Normal Rat Cortex*

Figures 1(a) and 1(b) illustrate the status of CBF before or after the administration of agents (saline or D-glucose) in a representative subject. In these ICT images with pseudo color, the regions with a higher level of blood flow are reddish, whereas the regions with lower velocity of blood flow are shown by blue. By comparing the images of two panels, it was apparent that as a result of the glucose administration, the CBF in the rat with acute hyperglycemia exhibited a distinct increase, peaking about 90–120 min after glucose injection, and finally recovered toward the baseline level [Fig. 1(b)].

The time courses of the relative changes in CBF for three vascular compartments [as drawn in Fig. 1(a)-0 min or 1(b)-0 min] are shown in Fig. 1(c) and 1(d), correspondingly. These curves in Fig. 1(c) show that the relative changes in CBF after saline administration at different vascular compartments had slight fluctuation over 180 min periods, ranging from 88 to 108% baseline value. Generally, we believe that normal CBF levels always vary from 80 to 120% baseline.

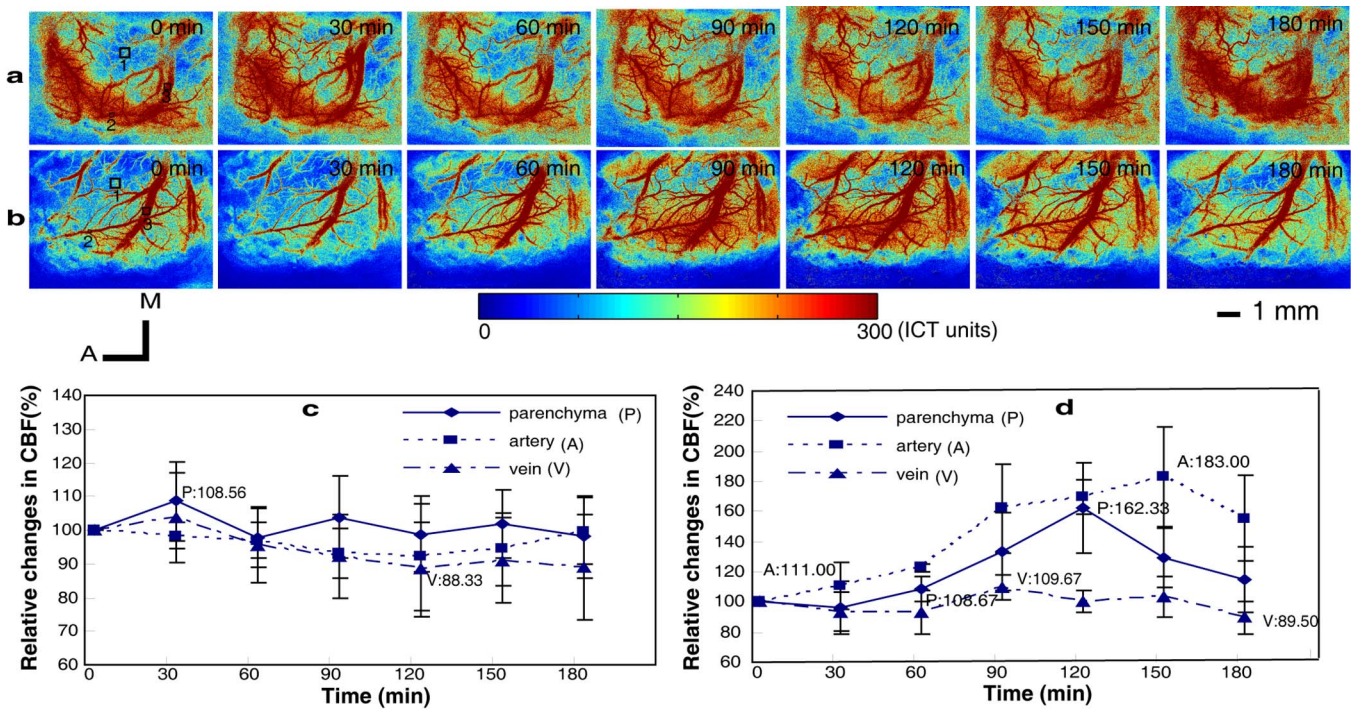

Fig. 1 The effect of acute hyperglycemia on CBF in a normal rat cortex. (a) The distribution of CBF perfusion was measured by laser speckle imaging before or after saline administration in a representative subject. (b) The distribution of CBF perfusion was measured by laser speckle imaging before or after glucose administration in a representative subject. Three vascular compartments (parenchyma, arteriole, and venule) (a)- 0 min or (b)- 0 min are indicated as three solid squares numbered 1, 2, and 3. (c) The time courses of saline-induced relative changes in CBF at the three vascular compartments are shown. (d) The time courses of glucose-induced changes in CBF at the three vascular compartments are denoted. Direction: A, anterior; M, median. Scale bar, 1 mm. All of the ICT images share the same direction and color bar. (Color online only.)

However, the relative changes of CBF during acute hyperglycemia had a significant difference among the various vascular compartments as shown by Fig. 1(d). First, the relative changes of CBF at the arteriole and parenchyma showed significant increase, but the amplitudes of the arterial response were larger than that of parenchyma. Second, the relative changes of CBF in the arteriole rise from 30 min (111% baseline), peaking at 150 min (183% baseline value). Increases in CBF at the parenchyma begin at 60 min (108% baseline value), peaking at 120 min (162% baseline value) after the glucose injection. Whereas no significant changes in CBF were detected at the venule, the relative changes of CBF varied from 89 to 108% baseline value [Fig. 1(d)].

3.2 *The Effect of Acute Hyperglycemia on CBF Following CSD in Rat Cortex*

The length of time for a wave to first appear in the imaged cortex was usually ~2 min after delivering electrical stimulation into the frontal cortex. CBF 30 min after each CSD wave was also monitored using laser speckle imaging. Figures 2(a) and 2(b) illustrate spatial and temporal changes in CBF following each CSD in the rats of the control group and the hyperglycemia group, respectively. Two series of ICT images in Fig. 2(a) and 2(b) were acquired at a 30-min interval. In the control group, CBF following each CSD was usually close to the baseline level during the experiment, as shown in Fig. 2(a); however, CBF following each CSD in the hyperglycemia rats had a stepwise reduction within 180 min after glucose injection [Fig. 2(b)].

Figures 2(c) and 2(d) show the time courses of the saline (glucose)-induced changes in CBF following CSD for all vascular components, as identified using the procedure illustrated in Fig. 1(c) and 1(d). From Fig. 2(c), we obtained that the relative changes of CBF in the three vascular compartments (parenchyma, arteriole, and venule), respectively, were 100–124, 96–124, and 95–108% of the baseline value, which were all close to the baseline level. There were no significant differences between them.

The relative changes of CBF following each CSD gradually fell off in the vascular compartments during acute hyperglycemia. These decreases in CBF for all compartments first appeared at 30 min after glucose administration and reached their minimums at 150 min. The minimums of relative changes were, respectively, 49, 56, and 60% of the baseline value [Fig. 2(d)]. There were not significant differences in relative changes of CBF among the various vascular compartments.

4 Discussion

It has been previously shown that CBF decreases due to acute or chronic hyperglycemia.[1] This phenomenon may provide useful information for the pathophysiology of ischemic stroke, suggesting possible mechanisms for the deleterious effects of hyperglycemia on cerebral injury due to ischemia. However, the methods used in the previous researches might have some limitations in spatial or temporal dimension, and these limitations might result in some details being neglected

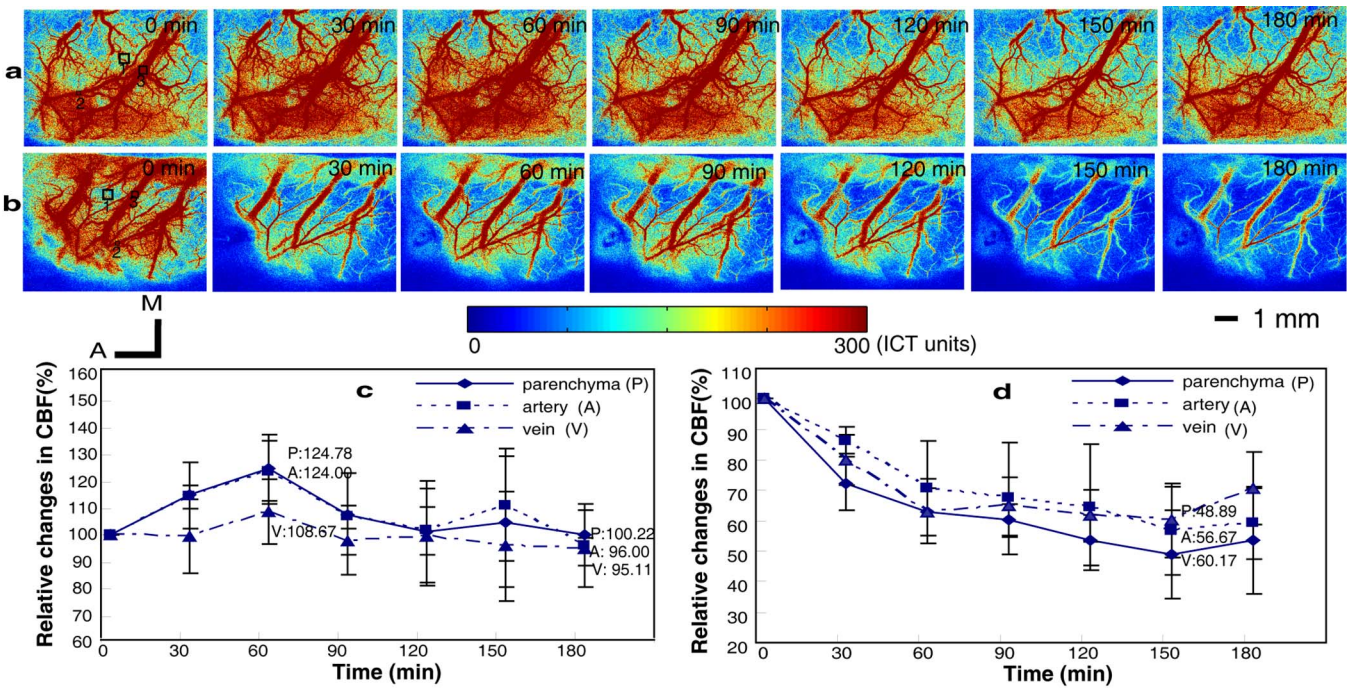

Fig. 2 The effect of acute hyperglycemia on CBF after CSD in rats. (a) The distribution of CBF perfusion was measured by laser speckle imaging before or after saline administration in a representative subject. (b) The distribution of CBF perfusion was measured by laser speckle imaging before or after glucose administration in a representative subject. Three vascular compartments (parenchyma, arteriole, and venule) in (a)-0 min or (b)-0 min are indicated as three solid squares with numbered 1, 2, and 3. (c) The time courses of saline-induced relative changes in CBF after CSD monitored at the selected region are shown. (d) The time courses of glucose-induced changes in CBF after CSD monitored at the region [(b)-0 min] are denoted. Direction: A, anterior; M, median. Scale bar, 1 mm. All of the ICT images share the same direction and color bar. (Color online only.)

in this phenomenon. Laser speckle imaging based on laser speckle temporal contrast analysis, as an advanced technique, can monitor the changes of CBF with high spatiotemporal resolutions, and this technique has been demonstrated in our previous works.[17,18] Here, we also used the technique for tracing the long-lasting effect of acute hyperglycemia on the CBF in normal cortexes or those with CSD, and thus to further explore the possible location of the vasculature where CBF is regulated.

We found that CBF over the normal rat cortex during acute hyperglycemia shows an extensive increase in this study. These results were also consistent with previous studies from systemic arteries which demonstrated that basal blood flow increased after 6-h hyperglycemia.[19] It is likely that the increase in CBF due to hyperglycemia was largely an effect of a tight coupling between cerebral metabolic rates of oxygen/glucose and CBF. The tight coupling was reportedly present in the resting brain, where high-level metabolic activity is accompanied with a high perfusion level of blood flow.[20] In general, full oxidation of glucose that produces carbon dioxide and water always occurs in the resting brain. During acute hyperglycemia, high plasma glucose could lead to an increase in cerebral metabolic rates of oxygen due to increases in consumption of oxygen to perform the full oxidation of glucose.[21] Thus, the raise in cerebral metabolic rates of oxygen could result in a distinct increase in CBF according to the tight coupling as described above, that is, increased blood flow in response to increased metabolism.

We also found that this increase of CBF related to acute hyperglycemia, as mentioned above, began at the arteriolar level and then was observed at the capillaries (parenchyma). Arterioles had the largest response, whereas the venous response was less evident. These results are in agreement with the activity-dependent changes of cortical blood volume in cats reported by Vanzetta et al.[22] Changes in cerebral blood volume displayed an apparent similarity with the CBF.[23] While the mechanism linked to the different changes in CBF at the all vascular components is still unknown, one potential mechanism may be related to the orientation of blood flow. Augmentation of CBF at the arteriole could lead to a passive increase in CBF at the downstream of the involved arteriole.

Our results also showed that in the normal rat, the relative changes in CBF following CSD at all vascular components were close to the baseline level. Previous studies have shown that a single episode of CSD is accompanied by reversible changes in CBF; it is characterized by a temporary CBF increase, followed by a long-lasting hypoperfusion in the cortex, terminated by a brief, small hyperperfusion,[8] and the duration of this reversible changes in CBF is within 15 ± 3 min.[24] In our experiments, the interval of 30 min between two CSD waves is sufficient for recovering the disturbance of CBF due to CSD.

Finally, we found that acute hyperglycemia causes simultaneous and gradual decreases in CBF following CSD throughout the vascular compartments. First, there have been reports asserting that most of the additional energy needed by activated brain area during CSD is provided by nonoxidative metabolism (glycolysis), although there is sufficient oxygen in the brain tissue. Meanwhile, hyperglycemia can also inhibit oxygen consumption in the active brain.[1] The two factors,

hyperglycemia and CSD, have cooperatively aggravated the fall of the oxidative metabolism rates, finally resulting in the decline of blood flow in the brain. Second, CSD is consistently associated with changes in the caliber of surface blood vessels, which was first reported by Leao.[25] Recently, Brennan et al.,[26] also reported there is significant dilatation of cortical surface arteriolar associated with CSD. Many different mechanisms of CSD-mediated arteriolar changes have been proposed.[26] Possible dilators include nitric oxide,[27] and parasympathetic innervation by acetylcholine,[28] and so on. Nakahata et al. noted that acute exposure toward hyperglycemia reduces cerebral vasodilatation produced by acetylcholine and related to nitric oxide synthases.[29] The long-lasting hypoperfusion among the disturbance of CBF due to CSD in the acute hyperglycemia group would be more difficult to recover than that in the control group because of a lack of effective cerebral vasodilatation. When a rat experienced acute hyperglycemia, its CBF presented gradual decreases after disconnected stimulation that evoked repetitive CSD.

These results also raise a question of why the vascular compartments showed stepwise reductions of CBF under this situation. Possible mechanisms could include impaired cerebrovascular autoregulation. It has been accepted that cerebrovascular autoregulation related to flow-pressure relationships play an important role in regulation of CBF. The autoregulation of CBF in the acute-hyperglycemia rats without CSD invasion was very good within a certain arterial pressure range, just as in normal rats.[30] However, cerebral vascular autoregulation of blood flow might be compromised as a result of hyperglycemia[31] accompanied with CSD invasion. This hypothesis is still awaiting future experimental confirmation.

The ability of hyperglycemia to aggravate cerebral ischemic damage has been well accepted,[32] whereas the underlying mechanisms are still not completely understood. Our results show that there are significant differences in the time course of changes in CBF during hyperglycemia between normal rats and those with CSD, suggesting that there may be a coeffective action between hyperglycemia and CSD on CBF, leading to the exacerbated outcome of ischemic stroke.

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References

1. A. L. McCall, "Cerebral glucose metabolism in diabetes mellitus," *Eur. J. Pharmacol.* **490**(1–3), 147–158 (2004).
2. W. L. Lanier, "The prevention and treatment of cerebral ischemia," *Can. J. Anaesth.* **46**(5 Pt 2), R46–56 (1999).
3. A. Bruno, J. Biller, H. P. Adams,Jr., W. R. Clarke, R. F. Woolson, L. S. Williams, and M. D. Hansen, "Acute blood glucose level and outcome from ischemic stroke: Trial of ORG 10172 in acute stroke treatment (TOAST) investigators," *Neurology* **52**(2), 280–284 (1999).
4. C. Bemeur, L. Ste-Marie, and J. Montgomery, "Increased oxidative stress during hyperglycemic cerebral ischemia," *Neurochem. Int.* **50**(7–8), 890–904 (2007).
5. J. K. Pomper, S. Haack, G. C. Petzold, K. Buchheim, S. Gabriel, U. Hoffmann, and U. Heinemann, "Repetitive spreading depression-like events result in cell damage in juvenile hippocampal slice cultures maintained in normoxia," *J. Neurophysiol.* **95**(1), 355–368 (2006).
6. S. Bahar, D. Fayuk, G. G. Somjen, P. G. Aitken, and D. A. Turner, "Mitochondrial and intrinsic optical signals imaged during hypoxia and spreading depression in rat hippocampal slices," *J. Neurophysiol.* **84**(1), 311–324 (2000).
7. H. Martins-Ferreira, M. Nedergaard, and C. Nicholson, "Perspectives on spreading depression," *Brain Res. Rev.* **32**(1), 215–234 (2000).
8. A. Gorji, "Spreading depression: a review of the clinical relevance," *Brain Res. Rev.* **38**(1–2), 33–60 (2001).
9. H. Cheng, Q. Luo, S. Zeng, S. Chen, J. Cen, and H. Gong, "Modified laser speckle imaging method with improved spatial resolution," *J. Biomed. Opt.* **8**(3), 559–564 (2003).
10. C. Ayata, H. K. Shin, S. Salomone, Y. Ozdemir-Gursoy, D. A. Boas, A. K. Dunn, and M. A. Moskowitz, "Pronounced hypoperfusion during spreading depression in mouse cortex," *J. Cereb. Blood Flow Metab.* **24**(10), 1172–1182 (2004).
11. C. Ayata, A. K. Dunn, O. Y. Gursoy, Z. Huang, D. A. Boas, and M. A. Moskowitz, "Laser speckle flowmetry for the study of cerebrovascular physiology in normal and ischemic mouse cortex," *J. Cereb. Blood Flow Metab.* **24**(7), 744–755 (2004).
12. Z. Wang, P. Li, W. Luo, S. Chen, and Q. Luo, "Peri-infarct temporal changes in intrinsic optical signal during spreading depression in focal ischemic rat cortex," *Neurosci. Lett.* **424**(2), 133–138 (2007).
13. T. Durduran, M. G. Burnett, G. Yu, C. Zhou, D. Furuya, A. G. Yodh, J. A. Detre, and J. H. Greenberg, "Spatiotemporal quantification of cerebral blood flow during functional activation in rat somatosensory cortex using laser-speckle flowmetry," *J. Cereb. Blood Flow Metab.* **24**(5), 518–525 (2004).
14. M. Atlan, B. C. Forget, A. C. Boccara, T. Vitalis, A. Rancillac, A. K. Dunn, and M. Gross, "Cortical blood flow assessment with frequency-domain laser Doppler microscopy," *J. Biomed. Opt.* **12**(2), 024019 (2007).
15. Z. Luo, Z. Wang, Z. Yuan, C. Du, and Y. Pan, "Optical coherence Doppler tomography quantifies laser speckle contrast imaging for blood flow imaging in the rat cerebral cortex," *Opt. Lett.* **33**(10), 1156–1158 (2008).
16. A. K. Dunn, H. Bolay, M. A. Moskowitz, and D. A. Boas, "Dynamic imaging of cerebral blood flow using laser speckle," *J. Cereb. Blood Flow Metab.* **21**(3), 195–201 (2001).
17. P. Li, S. Ni, L. Zhang, S. Zeng, and Q. Luo, "Imaging cerebral blood flow through the intact rat skull with temporal laser speckle imaging," *Opt. Lett.* **31**(12), 1824–1826 (2006).
18. W. Luo, Z. Wang, P. Li, S. Zeng, and Q. Luo, "A modified mini-stroke model with region-directed reperfusion in rat cortex," *J. Cereb. Blood Flow Metab.* **28**(5), 973–983 (2008).
19. J. A. Beckman, A. B. Goldfine, M. B. Gordon, and M. A. Creager, "Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans," *Circulation* **103**(12), 1618–1623 (2001).
20. B. Klein, W. Kuschinsky, H. Schrock and F. Vetterlein, "Interdependency of local capillary density, blood flow, and metabolism in rat brains," *Am. J. Physiol.* **251**(6 Pt 2), H1333–1340 (1986).
21. R. G. Shulman, F. Hyder, and D. L. Rothman, "Cerebral energetics and the glycogen shunt: Neurochemical basis of functional imaging," *Proc. Natl. Acad. Sci. U.S.A.* **98**(11), 6417–6422 (2001).
22. I. Vanzetta, R. Hildesheim, and A. Grinvald, "Compartment-resolved imaging of activity-dependent dynamics of cortical blood volume and oximetry," *J. Neurosci.* **25**(9), 2233–2244 (2005).
23. D. Malonek, U. Dirnagl, U. Lindauer, K. Yamada, I. Kanno, and A. Grinvald, "Vascular imprints of neuronal activity: relationships between the dynamics of cortical blood flow, oxygenation, and volume changes following sensory stimulation," *Proc. Natl. Acad. Sci. U.S.A.* **94**(26), 14826–14831 (1997).
24. N. Hadjikhani, M. Sanchez Del Rio, O. Wu, D. Schwartz, D. Bakker, B. Fischl, K. K. Kwong, F. M. Cutrer, B. R. Rosen, R. B. Tootell, A. G. Sorensen, and M. A. Moskowitz, "Mechanisms of migraine aura revealed by functional MRI in human visual cortex," *Proc. Natl. Acad. Sci. U.S.A.* **98**(8), 4687–4692 (2001).
25. A. A. P. Leão, "Spreading depression of activity in the cerebral cortex," *J. Neurophysiol.* **7**, 359–390 (1944).
26. K. C. Brennan, L. Beltran-Parrazal, H. E. Lopez Valdes, J. Theriot, A. Toga, and A. C. Charles, "Distinct vascular conduction with cortical

spreading depression," *J. Neurophysiol.* **97**, 4143–4151 (2007).
27. D. M. Colonna, W. Meng, D. D. Deal, and D. W. Busija, "Nitric oxide promotes arteriolar dilation during cortical spreading depression in rabbits," *Stroke* **25**, 2463–2470 (1994).
28. U. Reuter, J. R. Weber, L. Gold, G. Arnold, T. Wolf, J. Dreier, U. Lindauer, and U. Dirnagl, "Perivascular nerves contribute to cortical spreading depression-associated hyperemia in rats," *Am. J. Physiol.* **274**(6 Pt 2), H1979–1987 (1998).
29. K. Nakahata, H. Kinoshita, T. Azma, N. Matsuda, K. Hama-Tomioka, M. Haba, and Y. Hatano, "Propofol restores brain microvascular function impaired by high glucose via the decrease in oxidative stress. Laboratory investigations," *Anesthesiology* **108**(2), 269–275 (2008).
30. M. J. Rubin and H. G. Bohlen, "Cerebral vascular autoregulation of blood flow and tissue PO2 in diabetic rats," *Am. J. Physiol.* **249**(3 Pt 2), H540–546 (1985).
31. F. Pallas and D. F. Larson, "Cerebral blood flow in the diabetic patient," *Perfusion* **11**(5), 363–370 (1996).
32. M. W. Parsons, P. A. Barber, P. M. Desmond, T. A. Baird, D. G. Darby, G. Byrnes, B. M. Tress, and S. M. Davis, "Acute hyperglycemia adversely affects stroke outcome: A magnetic resonance imaging and spectroscopy study," *Ann. Neurol.* **52**(1), 20–28 (2002).