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Simultaneous imaging of cerebral partial pressure of oxygen and blood flow during functional activation and cortical spreading depression

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Abstract

We developed a novel imaging technique that provides real-time two-dimensional maps of the absolute partial pressure of oxygen and relative cerebral blood flow in rats by combining phosphorescence lifetime imaging with laser speckle contrast imaging. Direct measurement of blood oxygenation based on phosphorescence lifetime is not significantly affected by changes in the optical parameters of the tissue during the experiment. The potential of the system as a novel tool for quantitative analysis of the dynamic delivery of oxygen to support brain metabolism was demonstrated in rats by imaging cortical responses to forepaw stimulation and the propagation of cortical spreading depression waves. This new instrument will enable further study of neurovascular coupling in normal and diseased brain.

1. Introduction

The nature of the coupling between neuronal activity and the associated hemodynamic response is a subject of great debate [1–4]. Clearer understandings of the neuro-metabolic-vascular relationship will enable greater insight into the functioning of the normal brain and will also have significant impact on diagnosis and treatment of neurovascular diseases such as stroke [5,6], Alzheimer’s disease [7,8], and head injury [9]. In order to achieve this goal, simultaneous monitoring of the spatio-temporal characteristics of blood oxygenation, cerebral blood flow (CBF), and the cerebral metabolic rate of oxygen (CMRO₂) is crucial.

Positron emission tomography (PET) [10,11] and functional magnetic resonance imaging (fMRI) [12,13] are at present the most common neuroimaging modalities. Although these technologies are currently successfully applied for imaging brain hemodynamics and

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metabolism, each of them has specific limitations. For example, the spatio-temporal resolution of PET is limited, and fMRI requires careful calibration of the scaling factor between the blood oxygen level dependent (BOLD) signal and the relative changes of deoxy-hemoglobin concentration, as well as assumptions about the relationship between the changes in CBF and cerebral blood volume (CBV). In addition, PET and fMRI require multiple imaging methods and contrast agents and due to the cost and complexity they may not be readily available to researchers. On the other hand, optical imaging modalities have shown a great potential to provide high spatio-temporal resolution and more quantitative imaging of hemodynamic responses based on a variety of contrast mechanisms [14–19].

Simultaneous optical imaging of cortical hemodynamic parameters during functional activation and pathological conditions has been demonstrated previously in small rodents [16,20–23]. In particular, a single instrument [16] capable of simultaneously obtaining high-speed wide field images of relative cerebral blood flow (rCBF) based on laser speckle flowmetry [24–28] and hemoglobin oxygen saturation changes based on multi-spectral diffuse reflectance imaging [16,29] in the brain through a thinned skull preparation was successfully applied in studies of ischemia [30–32] and functional activation [24]. However, multi-spectral measurement of blood oxygenation relies on modeling the migration of photons through the brain and requires assumptions about the optical tissue parameters that may undergo dynamic changes during the experiment [32].

On the other hand, estimation of the partial pressure of oxygen (pO_2) based on oxygen dependent quenching of phosphorescence [33–36] should not be significantly affected by the changes in the optical parameters of the tissue and provides an absolute measure of pO_2 . Experimental systems that utilize oxygen sensitive dyes have been demonstrated in *in vivo* studies of the perfused tissue as well as for monitoring the oxygen content in tissue cultures, showing that phosphorescence quenching is a potent technology capable of accurate oxygen imaging in the physiological pO_2 range [19,37–44]. However, in most cases, their field of view was limited or their temporal resolution was low due to increased signal averaging.

In this paper we present a novel imaging instrumentation that provides high-speed wide-field images of pO_2 and rCBF in the brain vasculature by combining phosphorescence lifetime imaging with laser speckle contrast imaging. Efficient excitation of the oxygen sensitive phosphorescence dye (Oxypor R2, Gen 2 polyglutamic Pd porphyrin dendrimer [45,46]) with a pulsed laser and high light-collection efficiency allowed us to obtain high signal-to-noise ratio (SNR) images of the phosphorescence intensity decay. We demonstrate the potential of the system as a novel tool for quantitative analysis of the dynamic delivery of oxygen by imaging the pO_2 and rCBF during functional activation and cortical spreading depression (CSD) in rats. Future applications of this system should lead to a greater understanding of neurovascular coupling in normal and pathological brain conditions.

2. Methods

2.A. Animal Preparation

Sprague Dawley rats (250–320 g) were initially anesthetized with isoflurane and the femoral artery and vein were catheterized to monitor heart rate, blood pressure and blood gases, as well as for intravenous infusion. Body temperature was maintained at $37 \pm 0.1^\circ \text{C}$. Tracheotomy was performed and rats were ventilated with a mixture of air and oxygen. After completion of the surgery, isoflurane was discontinued and anesthesia was switched to alpha-chloralose (50 mg/kg intravenous bolus followed by 40 mg/(kg h) infusion). Imaging was performed through a $4 \times 4 \text{ mm}^2$ closed cranial window on the parietal bone. The dura was removed and the cranial window was filled with 1.5% agarose and sealed with a microscope coverslip. An additional 1 mm^2 burr hole on the frontal bone was used to induce CSD by intracortical microinjection

of KCl (10 μ l, 1 M). The phosphorescence probe Oxyphor R2 was injected via the femoral vein before imaging. All experimental procedures were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care.

2.B. Experimental Setup

The experimental setup is presented in Fig. 1. Imaging of both pO_2 and rCBF was performed with the same thermoelectrically cooled camera (Imager QE, La Vision). The camera frame rate was synchronized with the triggering rate of the pulsed laser (10 Hz) used for excitation of phosphorescence (Brilliant, Big Sky Laser Technologies, 532 nm wavelength, 180 mJ/pulse, 4.5 ns pulse duration). The light from the cranial window was collected by a low magnification infinity corrected objective (Olympus XL Fluor 4x/340, 0.28 numerical aperture, 29 mm working distance) and an image of $3.78 \times 2.86 \text{ mm}^2$ area was created on the CCD sensor by a 100 mm focal length tube lens. Ambient light and phosphorescence excitation light were suppressed with a 650 nm high pass filter.

For imaging of the partial pressure of oxygen the light from the pulsed laser was coupled into the multimode fiber and typically $5 - 10 \text{ mJ/cm}^2$ was delivered to the brain tissue at an angle of approximately 60 degrees with respect to the cranial window surface. The energy of the laser pulses was measured with a reference photodiode and used to correct for laser pulse energy fluctuations. For estimation of phosphorescence lifetimes, 4×4 binning of the CCD pixels was used and the CCD exposure time was set to 50 μ s. A sequence of 25 frames was acquired with variable delay times with respect to the laser Q-switch opening. The first frame (200 μ s before the laser pulse) was used to subtract the background light from the phosphorescence intensity images, and the delays of the remaining 24 frames were divided into three groups: initial 10 acquisitions with 5 μ s increments in delay following the laser pulse, followed by 10 acquisitions with 20 μ s increments in delay and 4 acquisitions with 400 μ s increments in delay. Example phosphorescence intensity images taken just after the laser pulse and 40 μ s and 130 μ s after the laser pulse are presented in Figs. 2(a), 2(b), and 2(c), respectively. Due to the shorter phosphorescence lifetime of the highly oxygenated arterial blood, the phosphorescence intensity from the arteries in these images (marked with an arrow on Fig. 2(a)) has a characteristically faster decay.

Based on the 24 consecutive frames of decaying phosphorescence intensity we calculated a two-dimensional (2D) map that represents a 2.4 s average of the pO_2 values. The calculation was performed by fitting the exponential decay lifetime τ of the phosphorescence intensity for each CCD pixel, followed by the conversion of phosphorescence lifetimes to the pO_2 values using an empirical Stern-Volmer-like relationship (*vide infra*). Single exponential decay of phosphorescence was assumed, and the fitting procedure was written in Matlab (MathWorks, Inc.) using a nonlinear least square fit with statistical weighting. Figure 2(d) shows phosphorescence intensity decay measurements and their fit from a single CCD pixel at point A marked with a cross on Fig. 2(a).

The phosphorescence lifetime of Oxyphor R2 ranges from a few tens to a few hundreds of microseconds for physiological pO_2 values in blood. Although the dye calibration was previously published for conditions that mimic pH, temperature, and albumin concentration of blood plasma [45], we found it important to perform *in vitro* calibration in the blood for the particular dye concentrations used in our experiments. In all animal experiments as well as for the calibrations we maintained $4 \times 10^{-5} \text{ M}$ concentration of Oxyphor R2 in the blood. In the blood, Oxyphor R2 binds to albumin, and the resulting complex serves as the *in situ*-formed oxygen probe. In this complex, the access of oxygen to the emitting chromophore is greatly attenuated compared to the unbound Oxyphor R2. Consequently, the phosphorescence lifetimes of unbound and albumin-bound probe at a given pO_2 level are significantly different.

At higher probe concentrations, some probe might exist in the unbound form, resulting in complex decay profiles and altered apparent calibration constants.

Accordingly, we took care to calibrate the lifetime relationship to pO_2 in blood. For calibration we prepared samples from sheep blood with different pO_2 values by exposing the blood to mixtures of O_2 , N_2 , and CO_2 . We assume that differences in albumin structure between mammalian species are insignificant with respect to their ability to shield the phosphorescent core of the R2 molecule from oxygen. The partial pressure of CO_2 and pH were maintained in the normal physiological range as confirmed by a blood gas analyzer (Rapid-lab 248, Bayer Healthcare LLC) and the temperature of the samples was kept constant at 37° C. The measurement procedure and fitting of the phosphorescence lifetimes for both the dye calibration and the animal experiments followed the identical procedure. Based on the calibration measurements, we expressed the relation between the pO_2 and phosphorescent lifetime with an empirical equation as $pO_2 = p_1 \exp(-\tau/p_2) - p_3\tau + p_4$ (Fig. 2(e)), and found $p_1 = 1098$ mmHg, $p_2 = 8.05$ μ s, $p_3 = 0.327$ mmHg/ μ s, and $p_4 = 75.4$ mmHg. This relation was then used in animal experiments. Probe R2 and all the other phosphorescent probes devised thus far do not exhibit ideal single-exponential decays, but rather provide non-uniform lifetime distributions. This is explained by the multitude of interactions of the probe molecules with albumin and other molecules in the blood. Fitting these complex decays by single-exponential functions – usually the most sensible way to treat the data at realistic noise levels, especially in imaging – results in so-called “apparent lifetimes.” Once inverted and plotted against pO_2 , these lifetimes yield somewhat non-linear Stern-Volmer plots. In-deed, the classic Stern-Volmer formula was derived assuming unique populations of emitting species and ideal single-exponential decays. A more comprehensive treatment would involve lifetime distribution analysis [47, 48]. In the original paper by Dunphy et al., the Stern-Volmer plot was obtained through measurements of lifetimes using a frequency-domain instrument operating in a single-frequency mode, with the frequency adjusted automatically throughout the measurement to maintain a constant phase-shift [45, 47]. In the time domain, this would be equivalent to taking just a selected part of the decay for the analysis to derive the apparent lifetime. If we were to do that, our plot would also be linear and similar to that reported by Dunphy et al. However, it works just as well to fit the entire data in the time domain and introduce correction for non-linearity into the resulting Stern-Volmer plot. Either method is perfectly valid for oxygen measurements. It should be mentioned that high probe concentrations in combination with high excitation flux levels should be avoided due to potential phototoxicity problems [38, 49], as oxygen quenching of luminescence is associated with formation of singlet oxygen and related phototoxic species [50].

Finally, Fig. 2(f) represents a 2D map of the partial pressure of oxygen that was obtained after fitting all individual pixels for the phosphorescence lifetime. The phosphorescence light diffuses through the optically scattering brain tissue and thus each CCD pixel represents an average of the microvascular compartments weighted towards the brain surface. Due to a much smaller volume and significantly higher oxygen quenching of phosphorescence in the arterial blood with respect to the venous blood, the obtained pO_2 values at locations distant from the major arteries largely represent venous blood oxygenation. This is convenient as estimation of oxygen metabolism is more strongly dependent on venous pO_2 .

Speckle imaging was performed after each 2.4 s sequence of phosphorescence imaging. CCD binning and exposure time were changed to 1×1 and 5 ms, respectively, and 5 consecutive frames were taken: one frame of the background light intensity and 4 frames during the laser exposure. Emission of the expanded laser diode light (Thorlabs, L808P200, 808 nm wavelength) was synchronized with the CCD exposure time. We calculated the speckle contrast at each CCD frame from a square of 15×15 surrounding pixels. Four consecutive speckle contrast frames were averaged and a 2D map that represents a 0.4 s average of the speckle

contrast was obtained. We converted the speckle contrast into an estimate of blood flow based on the maximum speckle contrast (0.15) of a static scattering sample in our system and the assumption that the velocity distribution of scatterers is Lorentzian [51]. The design of the detection optics was optimized for collection of the phosphorescence intensity and as a result it was sufficient but suboptimal for the speckle imaging. We then estimated rCBF by normalizing the flow by the baseline flow images. A single cycle of obtaining both pO₂ and blood flow images takes ≈ 4 s.

3. Results and Discussion

3.A. Imaging of Partial Pressure of Oxygen and Cerebral Blood Flow During Functional Activation

We demonstrated the capability of our system to provide real-time images of pO₂ and blood flow of a large (several millimeters) area of tissue by imaging functional activation of the cortex in rats. The animal forepaw was stimulated in a block design fashion, where one stimulation sequence consisted of a 1 s resting period, 15 s of stimulation, and a 14 s resting period. During stimulation, TTL pulses with 0.3 ms duration and 3 Hz repetition rate were sent to the stimulator amplifier and 2 mA pulses (50% above motor threshold) were applied to the forepaw. With respect to the experimental setup presented in Fig. 1, an additional computer was used in this experiment to generate the stimulation sequence and to record CCD control signals for later synchronization of the pO₂ and CBF measurements with the stimulus. The image acquisition was performed during several minutes and the results are presented in Fig. 3.

The position of the cranial window that exposes a portion of the forepaw cortical area and image of the cortical vasculature through the microscope eyepieces are presented in Fig. 3(a). The oxygen partial pressure map during the resting period of the stimulation sequence and the speckle contrast map are presented in Figs. 3(b) and 3(d), respectively. The composite Fig. 3(c) consists of the phosphorescence intensity gray image taken after the excitation laser pulse and a pO₂ activation color image. This pO₂ activation area was obtained by estimating the percent change in pO₂ during the stimulus, relative to baseline, and applying a 50% threshold. A more pronounced increase in pO₂ during activation seems to correlate with the position of the larger blood vessels (artery and vein) in the upper right corner of Fig. 3(c). Figure 3(e) shows rCBF and pO₂ values during several stimulation sequences, averaged over the image area marked with the squares on Figs. 3(b) and 3(d). The average pO₂ and rCBF responses obtained from the 6 stimulation sequences presented in Fig. 3(e) are presented in Fig. 3(f).

We also present in Fig. 3(f) calculated values of the relative cerebral metabolic rate of oxygen (rCMRO₂). CMRO₂ was estimated as a product of cerebral blood flow (CBF) and oxygen extraction fraction (OEF). By assuming 100% arterial blood oxygen saturation of hemoglobin, OEF was approximately expressed as $OEF = 1 - SvO_2$ and the venous oxygen saturation of hemoglobin (SvO₂) was calculated from the measured pO₂ values and the oxygen-hemoglobin dissociation curve [52, 53]. When calculating the SvO₂ we assumed that functional activation didn't affect the normal values of blood temperature, pH, and pCO₂. All relative values in Figs. 3(e) and 3(f) were calculated with respect to the average baseline value during the resting period.

The maximum increase of rCBF in Fig. 3(f), $\approx 36\%$, is in agreement with previously reported data obtained using optical Doppler methods [24, 54–56] and fMRI [57–59]. With laser Doppler flowmetry [54–56] and laser speckle flowmetry [24] the average peak increase in rCBF was between 14% and 60% for the durations of the forepaw stimulation between 4 s and 60 s. In fMRI studies the observed average peak increase in rCBF was 60% [57, 59] for the forepaw stimulations of 6 s and 30 s and 83% [58] for a 120 s long stimuli.

The average peak increase of pO_2 in Fig. 3(f), ≈ 5 mmHg, is in good agreement with the previous measurements of the pO_2 increase during forepaw stimulation [54, 56] that used point measurement of pO_2 based on oxygen quenching of phosphorescence. A peak increase of pO_2 of approximately 18% was obtained during stimulation of the visual cortex in cat using the same measurement technique [60].

We calculated a 13% average peak increase in $rCMRO_2$ during forepaw stimulation, well within the range of relative peak changes (5% – 25%) obtained in previous optical measurements of forepaw stimulation [24,55,61]. The average peak increase of the $rCMRO_2$, 24%, was reported in an fMRI study of forepaw stimulation [58]. The ratio of the relative peak $rCBF$ increase to the relative peak $rCMRO_2$ increase during activation was 2.8 in our measurement. Typical published values of this ratio using various measurement techniques are between 2 and 3 [24,61,62], but higher values of 3.5 [58] and ≈ 5 [63–65] have been reported, primarily with PET.

The temporal resolution of the system, ≈ 4 s, was not sufficient to resolve temporal differences in the rise times of pO_2 and $rCBF$ [24,54]. In the Conclusion, we describe a possible improvement to the system to enable better temporal resolution.

3.B. Imaging of Partial Pressure of Oxygen and Cerebral Blood Flow During Cortical Spreading Depression

Cortical spreading depression (CSD) is a self-propagating wave of cellular depolarization that travels with a speed of 2 – 4 mm/min through the cortex [66]. CSD has been implicated in migraine [67] and in progressive neuronal injury after stroke and head trauma [68]. Although first described in 1944 [69], the mechanism of CSD wave propagation and its impact on tissue metabolism and perfusion remain poorly understood [70] and it is the subject of intense research [71].

Figure 4(e) shows the position of the cranial window that was used for imaging and a microscope wide field image of the cortical vasculature. A CSD wave was initiated at the beginning of the experiment by injecting the KCl solution through the small opening in the frontal bone (Fig. 4(e)). The amount of injected KCl was sufficient to generate several CSD waves that were imaged for 10 minutes. Figure 4(a) represents the phosphorescence intensity map obtained 46 s after the start of the experiment. At that time a significant decay of pO_2 associated with the arrival of the first CSD wave is visible on the right side of Fig. 4(b). Figures 4(c) and 4(d) represent the speckle contrast and $rCBF$ values at the same time. Arrival of the first CSD wave in rats is usually associated with significant vasoconstriction and decreases in blood flow, as can be seen on the right side of Fig. 4(d).

Figure 4(f) represents the temporal evolution of pO_2 , $rCBF$, and $rCMRO_2$ averaged over the rectangular area that is marked in Figs. 4(b) and 4(d). The large drop in pO_2 of 15 mmHg, simultaneous with a less marked decrease in $rCBF$ upon arrival of the first CSD, can be clearly seen. These are followed immediately by significant increases in pO_2 (18 mmHg) and $rCBF$ [72]; the relative peak increase in $rCBF$ of 180% is in agreement with the previously reported value of 130% [72]. The absence of a pO_2 drop and the absence of a decrease in $rCBF$ at the arrival of the second and third CSD waves suggests that CSD-induced vasoconstriction plays a dominant role in creating the large pO_2 decrease at the arrival of the first CSD.

In contrast to functional activation measurements, imaging of the brain pathophysiology is usually associated with large changes in the physiological parameters of the brain. Significant changes in the optical scattering and absorption coefficients that are present during propagation of the CSD wave, while significant for spectroscopic and intensity-based measurements [32], should not considerably influence the phosphorescence lifetime-based pO_2 measurements.

However, transient changes in pH during CSD propagation [73–79] may be important to consider when estimating hemoglobin oxygen saturation from pO_2 for the estimate of $rCMRO_2$. In Fig. 4(f), the blue solid curve represents $rCMRO_2$ during the propagation of CSD waves, where we assumed a constant value of blood pH ($= 7.4$). When calculating the $rCMRO_2$ values that are represented with the dashed magenta curve in Fig. 4(f) we assumed a simplistic model of temporarily varying pH with the baseline pH $= 7.4$, which transiently decreased to 7.25 during CSD-induced increases in CBF. Although qualitatively similar and therefore both useful indicators of the brain tissue metabolism, in the absence of more precise knowledge about the venous blood pH during CSD wave propagation these two curves suggest a possible range of $rCMRO_2$ values.

We calculated a relative peak increase in $rCMRO_2$ of up to 100% during propagation of the first CSD assuming either a constant pH or variable pH (although variable pH exhibited a longer response). For subsequent CSDs, the peak increase is lowered to 50% and 80% for constant pH and variable pH, respectively. It is interesting to note that the peak time of $rCMRO_2$ significantly precedes the peak times of $rCBF$ and pO_2 in all CSD events, with more pronounced temporal difference present in the first CSD.

4. Conclusion

We developed a novel imaging technique that provides simultaneous 2D maps of the absolute value of pO_2 in the brain vasculature and $rCBF$ by combining phosphorescence lifetime imaging with laser speckle contrast imaging. The efficient excitation of the dye with laser pulses and usage of high light-collection efficiency optics allowed us to obtain high speed and high signal-to-noise ratio images of pO_2 even without an image intensifier. In addition, the phosphorescence lifetime measurement of pO_2 is largely insensitive to the changes in the optical parameters of the tissue during the experiment, which is usually a concern for the other optical imaging techniques that have a contrast mechanism based on intensity.

The capability of our system was demonstrated by imaging functional activation of the cortex in rats due to forepaw stimulation and by monitoring the propagation of CSD waves through the sealed cranial window. The current temporal resolution of the system of ≈ 4 s could be easily improved by using a separate CCD camera for the $rCBF$ imaging. This would eliminate the need to change the CCD acquisition parameters during the measurement and would also allow for optimal design of the speckle acquisition optics. In addition, our measurements suggest that a two-fold reduction in the number of CCD frames during the phosphorescence intensity decay does not significantly affect estimation of the lifetime, which may allow a temporal resolution for pO_2 estimation of ≈ 1 s. This may be sufficient to allow precise measurements of fast $CMRO_2$ changes during functional activation experiments where the difference in the rising times of blood flow and pO_2 is only a couple of seconds or less [16, 24,54].

The instrument has the potential to be a novel tool for quantitative analysis of the dynamic delivery of oxygen and the brain tissue metabolism that will lead to a greater understanding of neurovascular coupling in normal and diseased brain.

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Fig. 1.

Experimental setup. Two personal computers (PC1 and PC2) are controlling triggering signals and image acquisition. CCD - charge coupled device, BS - beam sampler, CL - collimating lens, FL - focusing lens, DAQ - data acquisition board.

Fig. 2.

Formation of the pO_2 image. (a), (b), and (c) Phosphorescence intensity images at different time delays in respect to the laser pulse. (d) Measurement and single exponential decay lifetime (τ) fit of the phosphorescence intensity at point A marked with the cross in (a). (e) In vitro calibration of $4 \times 10^{-5} \text{ M}$ blood concentration of Oxyphor R2. (f) pO_2 image was obtained based on dye calibration and calculated lifetime values at each CCD pixel. Arterial pO_2 measured during the experiment was 106 mmHg. Scale bar is 1 mm.

Fig. 3.

Imaging of the brain activation during forepaw stimulation. (a) Position of the cranial window and photograph of the cortical vasculature. (b) 2D pO₂ map during the resting period of activation sequence. (c) Composite image consisting of phosphorescence intensity (gray) and activation area (color). The activation area was obtained by estimating the percent change in pO₂ during the stimulus, relative to baseline, and applying a 50% threshold. (d) Speckle contrast image during the resting period of activation sequence. (e) Time courses of pO₂ (red solid curve) and rCBF (black dashed curve) during several stimulation sequences. Both pO₂ and rCBF values were averaged over the area marked with the windows in (b) and (d). Duration of the stimulus within each stimulation sequence was marked with the black horizontal bars in (e) and (f). (f) Average pO₂, rCBF, and rCMRO₂ responses during one stimulation sequence obtained from the 6 individual sequences in (e). Arterial pO₂ measured during the experiment was 102 mmHg. Scale bar is 1 mm.

Fig. 4.

Imaging of CSD. (a) and (b) Phosphorescence intensity image and pO_2 image at the arrival of the CSD ($t = 46$ s, marked with an arrow in (f)). (c) and (d) Laser speckle contrast image and $rCBF$ image at the arrival of the CSD (Media 1). (e) Positions of the cranial window and the location of KCl injection were marked on the schematic of the rat skull. Photograph of the cortical vasculature was taken through the microscope eyepieces. (f) Temporal evolutions of pO_2 (red solid line), $rCBF$ (black dashed line), and $rCMRO_2$ averaged over the rectangular area marked on (b) and (d). $rCMRO_2$ was calculated assuming a constant pH (blue solid line) and a variable pH (magenta dotted line) with transients centered around CSD induced peaks in $rCBF$. Arterial pO_2 measured at the beginning of the experiment was 81 mmHg. Scale bar is 1 mm.