Real-time tracking of brain oxygen gradients and blood flow during functional activation

Sang Hoon Chong,[a] **Yi Hong Ong**,[a,b] **Mirna El Khatib**,[c,d] **Srinivasa Rao Allu**,[c,d] **Ashwin B. Parthasarathy**,[e] **Joel H. Greenberg**,[f] **Arjun G. Yodh**,[a,*] **and Sergei A. Vinogradov**[c,d,*]

[a]University of Pennsylvania, Department of Physics and Astronomy, Philadelphia, Pennsylvania, United States
[b]University of Pennsylvania, Department of Radiation Oncology, Philadelphia, Pennsylvania, United States
[c]University of Pennsylvania, Perelman School of Medicine, Department of Biochemistry and Biophysics, Philadelphia, Pennsylvania, United States
[d]University of Pennsylvania, School of Arts and Sciences, Department of Chemistry, Philadelphia, Pennsylvania, United States
[e]University of South Florida, Department of Electrical Engineering, Tampa, Florida, United States
[f]University of Pennsylvania, Department of Neurology, Philadelphia, Pennsylvania, United States

Abstract

Significance: Cerebral metabolic rate of oxygen ($CMRO_2$) consumption is a key physiological variable that characterizes brain metabolism in a steady state and during functional activation.

Aim: We aim to develop a minimally invasive optical technique for real-time measurement of $CMRO_2$ concurrently with cerebral blood flow (CBF).

Approach: We used a pair of macromolecular phosphorescent probes with nonoverlapping optical spectra, which were localized in the intra- and extravascular compartments of the brain tissue, thus providing a readout of oxygen gradients between these two compartments. In parallel, we measured CBF using laser speckle contrast imaging.

Results: The method enables computation and tracking of $CMRO_2$ during functional activation with high temporal resolution (~7 Hz). In contrast to other approaches, our assessment of $CMRO_2$ does not require measurements of CBF or hemoglobin oxygen saturation.

Conclusions: The independent records of intravascular and extravascular partial pressures of oxygen, CBF, and $CMRO_2$ provide information about the physiological events that accompany neuronal activation, creating opportunities for dynamic quantification of brain metabolism.

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1 Introduction

Oxidative phosphorylation is the primary metabolic pathway by which the brain generates energy, and it requires uninterrupted delivery of oxygen to tissue.[1] Oxygen is carried by blood

*Address all correspondence to Arjun G. Yodh, yodh@physics.upenn.edu; Sergei A. Vinogradov, vinograd.upenn@gmail.com

and is distributed via a network of vessels, from which oxygen molecules diffuse along a concentration gradient set by the rate of oxygen consumption, known as the cerebral metabolic rate of oxygen ($CMRO_2$). Because there is minimal buffering of oxygen between the blood and respiring cells, cerebral blood flow (CBF) must rapidly respond to changes in neuronal activity. The oxygen concentration gradients are reflected by the vascular versus tissue (mitochondrial) partial pressures of oxygen (pO_2); thus, they encode information about oxygen consumption and supply as well as information about changes in $CMRO_2$ and vascular responses.

Quantification of $CMRO_2$ has been a longstanding goal in neuroscience. In a steady state, $CMRO_2$ can be a useful marker of tissue pathology, such as in stroke,[2] traumatic brain injury,[3] and cancer.[4] By contrast, dynamic measurements of $CMRO_2$ during neuronal activation provide information about the brain metabolic events that underlie functional responses.[5] Positron emission tomography (PET) with ^{15}O-labeled compounds is arguably the most established method for $CMRO_2$ measurements in a steady-state.[6] PET can be used in humans, but quantitative PET requires independent measurements of CBF and the total cerebral blood volume (CBV), and it relies on complex multiparametric models for the calculation of $CMRO_2$.[7] Furthermore, the temporal resolution of PET is insufficient for tracking metabolic dynamics during neuronal activation.[8]

For dynamic tracking of brain responses, the most widely used techniques are blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI)[9] and functional near infrared spectroscopy (fNIRS).[10,11] In both methods, $CMRO_2$ is derived from hemoglobin oxygen saturation, and its quantification requires independent measurements of oxygen extraction fraction, CBF, and CBV.[12–16] As a result, BOLD fMRI and fNIRS are best suited for probing relative changes in $CMRO_2$, and the resultant $CMRO_2$ dynamics are inherently tied to the dynamics of CBF. However, $CMRO_2$ and CBF may not change in the same way in response to activation, and their timing carries valuable information about the dynamics of neurovascular coupling[17] that is inaccessible to BOLD fMRI and fNIRS.

$CMRO_2$ can also be inferred from measurements of lateral gradients of oxygen around individual vessels coupled with diffusion-based models, such as the Krogh-Erlang cylinder model.[18] pO_2 gradients around vessels were first measured with oxygen microelectrodes.[19] More recently, two-photon phosphorescence lifetime microscopy (2PLM)[20] has proven superior for spatially resolved pO_2 measurements. The 2PLM method is minimally invasive and capable of probing pO_2 at multiple locations near vessels in the brain.[21,22] However, oxygen mapping by 2PLM requires long observation times, and hence it is currently used only for gradient measurements in steady-states.[23,24] Nevertheless, as per this work, phosphorescence lifetime oximetry has been successfully used to measure stimulus-induced changes in brain pO_2[25] at speeds comparable to transients of neuronal activity. Additionally, the laser Doppler method and oxygen microelectrodes have been used concurrently during functional stimulation to track local CBF and intravascular and extravascular pO_2 (p_iO_2 and p_eO_2) in selected locations, but $CMRO_2$ was not computed.[26]

Herein, we introduce and demonstrate an all-optical approach for dynamic measurements of $CMRO_2$. The new methodology is based on quantification of oxygen concentration in the brain using two macromolecular phosphorescent probes, Oxyphors PtG4 and PtR4, placed separately in the intravascular and extravascular compartments. The Oxyphors do not diffuse across the blood brain barrier and have distinguishable optical spectra. Their optical signals can be obtained independently and concurrently for direct measurement of oxygen gradients between the compartments. In our work, we sampled oxygen gradients in rat brain cortex at a rate of ~7 Hz, but this sampling frequency can potentially be increased more than 10-fold. In parallel, we measured local CBF by laser speckle contrast imaging (LSCI). Overall, the obtained data enabled us to correlate changes in $CMRO_2$ with changes in CBF and thereby ascertain the timing and relative magnitudes of the physiological events that accompany neuronal activation. The new approach is minimally invasive, offers superior time-resolution (potentially approaching milliseconds), and creates novel opportunities for dynamic tracking and quantification of absolute $CMRO_2$ and neurovascular coupling dynamics.

2 Materials and Methods

2.1 Two-color Phosphorescence Lifetime Oximetry

The phosphorescent probes used in this study were Oxyphors PtG4 and PtR4. PtG4 has been used previously.[27–29] PtR4 was synthesized specifically for this work (Supplementary Material, p. S3). PtR4 is similar to the probe PdR4,[30] except that the central metal ion in the porphyrin is Pt(II) and the shell surrounding the porphyrin in PtR4 is composed of mixed arylglycine/glutamate dendrons. The difference between Pt and Pd porphyrin-based probes has been discussed previously.[31] The synthesis and the photophysical data for PtR4 can be found in the Supplementary Material (pp. S3–S9). The probes were calibrated using a setup described previously.[30]

The instrument for two-color phosphorometry was constructed in-house [Fig. 1(a)]. The light sources for excitation of PtG4 and PtR4 were modulated diode lasers (Power Technology) operating at $_{max}$ ¼ 630 nm (15 mW) and $_{max}$ ¼ 517 nm (10 mW), respectively. Both diode lasers have a rise time of ~50 ns. Avalanche photodiodes (APDs) were employed for light detection

Fig. 1 (a) Scheme of the experimental setup. (b) Representative wide-field image of the craniotomy window over the somatosensory area. Scale bar: 1 mm. The full LSCI imaging area is shown by the dashed square. The solid white square (200 m × 200 m) indicates the region used for LSCI data processing, which coincided with the focus spot of the phosphorometer lasers (~200 m in diameter). This spot was located ~300 m away from the probe injection site (seen as a greenish area). (c) Optical absorption and phosphorescence spectra of Oxyphors PtG4 (channel 1) and PtR4 (channel 2). The laser lines (635 and 517 nm) of the respective channels and the LSCI laser line (852 nm) are shown by vertical bars. The absorption spectra are scaled by the respective extinction coefficients [$_{620}$(PtG4) ≈ 1.0×10^5 M^{-1} cm^{-1} and $_{510}$(PtR4) ≈ 2.0×10^4 M^{-1} cm^{-1}]. The emission spectra are scaled such that their areas are proportional to the probes' phosphorescence quantum yields ($_{PtG4}$ ¼ 0.067 and $_{PtR4}$ ¼ 0.052). The emission wavelength ranges isolated by the optical filters and seen by the detectors in Ch1 (PtG4) and Ch2 (PtR4) are shown by the shaded areas.