High-speed quantitative optical imaging of absolute metabolism in the rat cortex

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Abstract

Significance: Quantitative measures of blood flow and metabolism are essential for improved assessment of brain health and response to ischemic injury.

Aim: We demonstrate a multimodal technique for measuring the cerebral metabolic rate of oxygen (CMRO₂) in the rodent brain on an absolute scale (µMO₂/min).

Approach: We use laser speckle imaging at 809 nm and spatial frequency domain imaging at 655, 730, and 850 nm to obtain spatiotemporal maps of cerebral blood flow, tissue absorption (µₐ), and tissue scattering (µₛ₀). Knowledge of these three values enables calculation of a characteristic blood flow speed, which in turn is input to a mathematical model with a “zero-flow” boundary condition to calculate absolute CMRO₂. We apply this method to a rat model of cardiac arrest (CA) and cardiopulmonary resuscitation. With this model, the zero-flow condition occurs during entry into CA.

Results: The CMRO₂ values calculated with our method are in good agreement with those measured with magnetic resonance and positron emission tomography by other groups.

Conclusions: Our technique provides a quantitative metric of absolute cerebral metabolism that can potentially be used for comparison between animals and longitudinal monitoring of a single animal over multiple days. Though this report focuses on metabolism in a model of ischemia and reperfusion, this technique can potentially be applied to far broader types of acute brain injury and whole-body pathological occurrences.

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1 Introduction

Assessing brain metabolism on a quantitative scale is critical for improved diagnosis, monitoring, and treatment of a wide variety of acute brain injury caused, for example, by ischemia, hemorrhage, and trauma. Indeed, numerous studies show abnormal brain metabolism in such
急性脑损伤在患者中。测量脑氧代谢率（CMRO2）可能提供有关脑组织损伤后的信息，特别是在特定条件下，知识CMRO2在绝对单位中可以消除基线测量的需要，并且有助于跟踪长期的脑恢复。实际上，在临床环境中，急性脑损伤的情况下，很难获得基线（预损伤）测量，这强调了需要建立绝对而不是相对的测量标准。绝对CMRO2测量将使我们能够定量比较不同受试者的不同值。

不幸的是，常规的临床监测技术（例如，动脉血压监测、脉搏氧饱和度监测、激光频谱流速计，以及功能性核磁共振成像）不能单独测量脑代谢的改变。现有技术在血流速度变化方面的应用非常有限。

2.1 动物准备

在动物准备方面，所有实验都是在遵守动物实验伦理委员会（IACUC）规定的条件下进行的，该委员会位于加州大学欧文分校。在实验前，所有实验对象都被麻醉并进行气管插管。每个受试者都在头骨上放置电极，用于皮层电图（ECoG）和部分切除的右侧（4 mm）。

2.2 动物切除

我们使用SFDI和LSI技术来测量血液流动和血红蛋白含量，并且在多个波长下，我们能够同时测量散射性变化。这些技术的结合能够以高空间和时间分辨率来成像血液流动和血红蛋白变化。

SFDI和激光频谱成像（LSI）可以量化组织代谢变化，具有高空间和temporal resolution。11 我们最近应用了高速LSI12和SFDI8来测量血流、氧合，以及在预临床心脏骤停（CA）模型中的组织散射。

Wilson et al.: 高速定量光学成像在大鼠皮层绝对代谢中的应用
anterior-to-posterior) was performed to enable imaging of a portion of the right sensory and visual cortices. Cannulation of the femoral artery allowed the delivery of drugs, sampling of blood, and monitoring of blood pressure.

### 2.2 Cardiac Arrest and Cardiopulmonary Resuscitation

Figure 1 shows the multimodal setup employed in the experiments. At the onset of each experiment, the level of isoflurane was decreased from 2% to 0.5% to 1.0%. Concurrently, the mixture of inhaled gases was altered from 50% O2 + 50% N2 to 100% O2. Two minutes later, to reduce confounding effects of isoflurane on cerebral perfusion and metabolism, the anesthesia was turned off, at which time the subject breathed room air (21% O2). During this same period, 1 mL of 2 mg/kg Vecuronium (a neuromuscular blocker) and 1 mL of heparinized saline were administered intravenously, which led to respiration controlled solely by the ventilator. This stage of the experiment lasted for 3 min, after which the ventilator was turned off to induce asphyxia, leading to progressive hypoxic hypercapnic hypotension. CA was defined as the period over which the pulse pressure was below 10 mmHg and systolic pressure below 30 mmHg. The conditions of these experiments induced pulseless electrical activity, which is common in CA patients in a hospital setting.

Forty-five seconds before the end of the CA period, the ventilator was turned on (respiratory rate = 85 breaths/min, PIP = 17.5 to 18.5 cm H2O, PEEP = 3 cm H2O at 2.5 LPM), and 100% oxygen was delivered. Immediately before the onset of cardiopulmonary resuscitation (CPR), 0.01 mg/kg epinephrine, 1 mmol/kg sodium bicarbonate, and 2 mL of heparinized saline were administered intravenously. Then, CPR was performed via external cardiac massage and terminated upon return of spontaneous circulation (ROSC), as identified from arterial blood pressure measurements. Subsequently, the animal was monitored continuously with arterial blood pressure, optical imaging, and ECoG for an additional ~2 h, after which the animal was euthanized with pentobarbital. As a quantitative measurement of the information...
content contained in the ECoG signals, we calculated an entropy-based parameter known as ECoG information quantity (IQ). Recovery of ECoG signal following ROSC was quantified by (1) time to initial resumption (burst) of ECoG activity and (2) ECoG IQ 90 min post-ROSC (as in Ref. 19).

2.3 Laser Speckle Imaging

For LSI, an 809-nm laser with long coherence length (Ondax, Monrovia, California) served as the light source. To increase uniformity of illumination over the imaged region of interest (ROI), a ground-glass diffuser (ThorLabs, Inc., Newton, New Jersey) was placed between the laser and the brain. A CCD camera (Point Grey Research Inc., Richmond, BC, Canada) detected the back-scattered light with a 10-ms exposure time, resulting in image acquisition at a frame rate of 60 Hz. Using a 5 × 5 sliding spatial window filter, the equation \( K = \sigma / \langle I \rangle \) was employed to calculate the local speckle contrast \( K \) at each pixel, where \( \langle I \rangle \) is the mean intensity within the filter and \( \sigma \) is the standard deviation within the filter.\(^{20}\) Then, the speckle flow index (SFI) was determined from the values of \( K \) and the exposure time \( T \), via a simplified speckle imaging equation \( \text{SFI} = 1/(2TK^2).\)\(^{20}\) Time-resolved SFI curves were generated by taking the mean of the SFI over a selected ROI at each time point.

2.4 Spatial Frequency-Domain Imaging

For SFDI, light-emitting diodes (LEDs) of three different wavelengths (655, 73, and 850 nm) were used as light sources. The light was directed to a spatial light modulator that projected square-wave patterns onto the brain.\(^{8}\) Backscattered light was captured using a scientific complementary metal-oxide-semiconductor (sCMOS) camera (Hamamatsu Photonics). An Arduino Due microcontroller board was used to synchronize the camera acquisition, spatial light modulator, and LEDs. For each wavelength, four patterns were projected onto the tissue in sequence. The first pattern was nonmodulated (i.e., DC illumination), and the three subsequent patterns were modulated at spatial frequency \( \sim 0.3 \text{ mm}^{-1} \) with three distinct spatial phases to enable demodulation.\(^{21}\) Thus, there were a total of (3 wavelengths \( \times 4 \) frames) = 12 frames of SFDI data for each measurement time point. The detected square wave pattern could be approximated as a sinusoid, allowing demodulation in the manner described previously by our group.\(^{22}\)

With this acquisition scheme, we were able to reconstruct tissue hemodynamics and CMRO\(_2\), at an effective imaging rate of \( \sim 14 \text{ Hz} \).

After demodulating the spatially modulated data, the diffuse reflectance at each time point and wavelength was calculated from the raw data via calibration against a tissue-simulating phantom with known optical properties.\(^{23}\) The diffuse reflectance maps were then fit with a Monte Carlo model to extract the tissue absorption coefficient \( \mu_a \) and reduced scattering coefficient \( \mu_s' \) at each wavelength.\(^{8}\) Next, the average \( \mu_s' \) was determined for a selected ROI and a new \( \mu_s \) determined using diffuse reflectance with the nonmodulated pattern and this average \( \mu_s' \).

To calculate the concentrations of oxygenated and deoxygenated hemoglobin (ctHbO\(_2\) and ctHb, respectively) within the tissue, this new \( \mu_s(\lambda) \) spectrum was fit with the model spectrum \( \mu_s(\lambda) = 2.303(\text{ctHbO}_2\epsilon_{\text{HbO}_2} + \text{ctHb}\epsilon_{\text{Hb}}) \), where \( \epsilon_{\text{HbO}_2} \) and \( \epsilon_{\text{Hb}} \) were the molar extinction coefficients of oxy- and deoxyhemoglobin, respectively. The total tissue hemoglobin concentration (ctHb\(_{\text{tot}}\)) was calculated by summing ctHb and ctHbO\(_2\). The tissue oxygen saturation was determined using the equation \( \text{StO}_2 = \text{ctHbO}_2/(\text{ctHbO}_2 + \text{ctHb}) \).

2.4.1 Correction of speckle flow index for tissue absorption and scattering

\( K \), and hence SFI, depends on local optical properties.\(^{23}\) To correct the measured SFI for dynamic optical properties, the measured \( K \) values were converted to a characteristic flow speed \( (v_c) \) by using the following equation:\(^{23}\)

\[
K^2 = \frac{\left( \frac{2}{\pi} \int_0^\infty \beta G_i^2(\tau)(1 - \tau/T)d\tau \right)}{G_i^2(\tau = 0)},
\]

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\( \beta \) is a constant (typically set to 1) related to polarization and coherence properties of the LSI instrumentation. From the Siegert relationship, the intensity autocorrelation function \( G_1(\tau) \) is related to \( G(\tau) \), which, in turn, is described by the correlation diffusion equation:

\[
\nabla^2 G_1(\tau) - \mu_{\text{eff}} G_1(\tau) = q.
\]

In Eq. (2), \( q \) is the source term; and \( \mu_{\text{eff}} = (3\mu_{\text{a, dyn}}\mu_{\text{a}})^{1/2} \), where \( \mu_{\text{a}} = (\mu_{\text{a}} + \mu_{\text{a}}^c) \) is the tissue transport coefficient and \( \mu_{\text{a, dyn}} = (\mu_{\text{a}} + \mu_{\text{a}}^c k_0^2(\Delta^2(\tau))/3 \) the dynamic tissue absorption coefficient.\(^{23} \) In the equation for \( \mu_{\text{a, dyn}} \), \( (\Delta^2(\tau)) \) is the mean square displacement of the moving scatterers (i.e., the red blood cells) and \( k_0 \) is the photon wavenumber. Solving Eq. (2), \( G_1(\tau) \) can be written as

\[
G_1(\tau) = \frac{3P_o A \mu_{\text{a}}(\mu_{\text{a}} + 1)}{(\mu_{\text{a}} + 3A)}. \tag{3}
\]

In Eq. (3), \( P_o \) is the incident optical power, and \( A \) is a function of the tissue refractive index.\(^{21} \) All other terms in Eq. (3) are exclusively functions of the static and dynamic tissue absorption and scattering coefficients \( (\mu_{\text{a}}, \mu_{\text{a}}^c, \mu_{\text{a, dyn}}) \). \( \mu_{\text{a, dyn}} \) is a function of \( (\Delta^2(\tau)) \), and \( (\Delta^2(\tau)) \) is related to the characteristic flow speed \( v_c \); via the equation \( (\Delta^2(\tau)) = v_c^2 \) (for directional flow).\(^{24} \) Using this framework and inputting the measured value of \( K \) from LSI and the measured \( \mu_{\text{a}} \) and \( \mu_{\text{a}}^c \) from SFDI at each time point, Eq. (1) was solved for \( v_c \) at each time point and each pixel by iterating over a predefined grid of potential \( v_c \) values and minimizing a least-squares cost function. The resulting spatiotemporal values of \( v_c \) were used in place of SFI in the subsequent steps to achieve an optical property-corrected calculation of CMRO\(_2\).

### 2.4.2 Absolute cerebral metabolic rate of oxygen calculation

To calculate absolute CMRO\(_2\), we start from the equation:\(^{25} \)

\[
\text{CMRO}_2 = (\text{CBF})/(\text{OEF})/[O\text{2}]_a. \tag{4}
\]

In Eq. (4), CBF is the cerebral blood flow, \([O\text{2}]_a\) is the arterial concentration of oxygen, and OEF is the oxygen extraction fraction, equal to \([O\text{2}]_v - [O\text{2}]_a)/[O\text{2}]_a\), where \([O\text{2}]_v\) is the venous concentration of oxygen. For a single arteriole, \( (\text{OEF})/(O\text{2})_a\) represents the molar concentration of oxygen that was extracted from that arteriole and used by the brain for metabolic processes related to the synthesis of ATP. This quantity is equivalent to the molar concentration of deoxygenated hemoglobin that arrives in a nearby venule following oxygen extraction by the brain. Therefore, within our measurement paradigm,\(^{11} \) Eq. (4) is rewritten as

\[
\text{CMRO}_2 = 4a(v_c)(\text{ctHb}_v/(\text{Hb}_v/[\text{ctHb}_v]_p)). \tag{5}
\]

In Eq. (5), \text{ctHb}_v is the tissue concentration of deoxygenated hemoglobin in an ROI atop a large vein in the ctHb maps obtained from SFDI. The factor of 4 accounts for the fact that the hemoglobin molecule has four binding sites for oxygen. Since \( v_c \) is a characteristic flow parameter and not an absolute value of blood flow, it is necessary to include the proportionality constant \( a \) in the equation to convert \( v_c \) into a quantity with units of absolute flow speed. The factor \( (\text{Hb}_v/[\text{ctHb}_v]_p) \) accounts for partial-volume effects caused by the diffuse nature of light propagation in the brain. Equation (4) requires an intravascular oxygen concentration, but SFDI measures a bulk tissue deoxyhemoglobin concentration. Hence, a blood-volume fraction term is required to convert between these two quantities. The numerator, \( \text{Hb}_v \), is the concentration of hemoglobin in the blood sampled from the femoral artery of the animal during the arterial blood gas (ABG) measurement. The denominator, \( (\text{ctHb}_v)_p \), is the mean total tissue hemoglobin concentration in the parenchyma during the period that the ABG was acquired. The factor \( (\text{Hb}_v/[\text{ctHb}_v]_p) \) enables the required conversion of our optical ctHb measurements from the scale of a tissue hemoglobin concentration to the scale of a vascular hemoglobin concentration.
concentration, mitigating the partial volume effect and allowing us to measure CMRO₂ on an absolute scale.

The parameter α is typically unknown; thus, the quantity reported in optical brain imaging studies is usually the relative CMRO₂ (rCMRO₂). However, in this report, we were able to measure absolute CMRO₂ using a “zero-flow” boundary condition,²⁶ which is provided by the onset of global cerebral ischemia in our animal model:

\[
4\alpha(v_c)|_{T_{\text{pre}}} (\text{ctHb}_v)|_{T_{\text{pre}}} = 4(\text{dctHb}_v/\text{dt})|_{T_{\text{post}}}. \tag{6}
\]

In Eq. (6), the vertical bars denote the time at which the variable was evaluated. This procedure was performed for each of the 10 subjects in this study, using the values of SFI and ctHbₐ immediately before asphyxia (T_{pre}) and the mean rate of change dctHbₐ/dt immediately after the onset of asphyxia (T_{post}). The value of dctHbₐ/dt was measured by fitting a sigmoid function to the ctHb curve during the beginning of the “zero-flow” period, finding the t$_{50}$ value of the sigmoid, linearizing the sigmoid within a 30-s window centered on the t$_{50}$ point, and calculating the slope of the resulting line segment. The values of α and absolute CMRO₂ then were calculated over the entire craniectomy region at each measurement time point.

3 Results

Immediately following CA, cerebral hemodynamics are spatially heterogeneous (Fig. 2). For example, the rate of change in cerebral ctHb following the start of asphyxia was decreased in the veins compared to the parenchyma.

![Figure 2](https://ebooks.spiedigitallibrary.org/journals/Neurophotonics)
58.3 ± 32.3 μM/min in an ROI selected over a large vein [Fig. 2(b)], but only 33.6 ± 13.6 μM/min in an ROI selected over the parenchyma.

Maps of absolute CMRO₂ throughout a representative CA/CPR experiment are shown in Fig. 3. At baseline, the animal is under anesthesia (2% isoflurane). After 2 min of anesthesia washout, the CMRO₂ increased by a factor of ~2 as the subject woke up. Following the onset of ischemia, the CMRO₂ rapidly decreased as the subject entered CA. After resuscitation, the CMRO₂ rapidly increased until reaching a maximum value at ~8 min post CPR (during hyperemia). Subsequently, the CMRO₂ decreased toward baseline as cerebral electrical activity resumed (~12 min post-CPR).

![Fig. 3](image)

**Fig. 3** Absolute CMRO₂ (μM O₂/min) maps of a ~6 mm × 4 mm region of the rat brain at different time points during a CA/CPR experiment. Metabolism activity increases as anesthesia is being washed out (between "baseline" and "start ischemia"), followed by a sharp decrease during ischemia. Following CPR, CMRO₂ recovers to anesthesia-free baseline level (3 min post-CPR), subsequently increases to values higher than baseline (5 to 8 min post-CPR), and then declines to values approaching anesthetized baseline level once cerebral electrical activity resumes (12 min post-CPR). Large vessels (dark blue) have been removed from the CMRO₂ images to signify that the oxygen metabolism we are measuring is occurring in the parenchyma.

![Fig. 4](image)

**Fig. 4** Optical properties affect calculation of CMRO₂. Comparison of SFI (light blue), CMRO₂ calculated using SFI (uncorrected CMRO₂, dark blue), and CMRO₂ calculated using \( v_e \) (corrected CMRO₂, red), which accounts for the effects of tissue optical properties on SFI. For ease of comparison, the three curves are normalized to their value at a point near the end of the washout period (t ~ 4 min). This correction reveals differences in the observed rates of change in CMRO₂ during reperfusion and resumption of ECoG bursting, suggesting the need to take optical properties into account even for relative CMRO₂ measurements.
Calculation of changes in CMRO$_2$ is affected by optical properties (Fig. 4). Optical properties measured with SFDI, along with Eq. (1), enable calculation of a characteristic flow speed ($v_c$) that can be used in place of SFI for the calculation of relative CMRO$_2$ (rCMRO$_2$). During each of the experimental phases, a comparison of rCMRO$_2$ trends suggests that the metabolic activity is at times greater (i.e., during the hyperemic phase) and lower (i.e., during CA) with use of SFI instead of $v_c$ in the calculation of CMRO$_2$. This result demonstrates that changes in cerebral optical properties can affect calculations of CMRO$_2$ dynamics using SFI alone to measure flow and hence illustrates the need to measure optical properties to properly characterize cerebral metabolic activity.

Figure 5 shows distributions of CMRO$_2$ values measured with our imaging setup as compared to values reported in the literature using various medical imaging approaches, including magnetic resonance methods (MRI/MRS)$^{27-29}$ and PET.$^3$ The LSI + SFDI method reported here measures absolute CMRO$_2$ values that are within the range measured with these approaches, suggesting the accuracy of our optical imaging approach to determine CMRO$_2$. It is important to note that in Fig. 5, each data point in the boxplot for our method represents an individual rat, but in the MRI/MRS and PET boxplots, each data point is the mean CMRO$_2$ value over the group of rats used in each of the studies cited above. Therefore, it is not surprising that there is a greater spread in the CMRO$_2$ values in Fig. 5 that were obtained from our method.

**4 Discussion**

Here, we provide, to the best of our knowledge, the first demonstration of dynamic imaging of absolute CMRO$_2$ in the living brain using a combination of LSI and SFDI techniques. We use the tissue optical properties measured with SFDI to account for their effects on interpretation of the LSI information. We then use the “zero-flow” condition inherent in our CA experimental paradigm to solve for the coefficient $\alpha$ in the CMRO$_2$ equation using a continuity condition at the boundary between normal flow and zero-flow states. Using this technique, we perform quantitative spatial mapping of absolute CMRO$_2$ continuously throughout the different stages of the CA + CPR experiment. The CMRO$_2$ obtained from our optical system agreed well with established brain imaging techniques (PET, MRI/MRS).
This paradigm for measuring absolute CMRO$_2$, in units of $\mu$MO$_2$/min, enables direct comparison of metabolic activity among subjects, across separate imaging sessions, and on different days for a single subject. This approach potentially enables longitudinal monitoring of cerebral recovery for days or weeks following ischemia and reperfusion. The methods described here can be applied to quantitative measurement of metabolic recovery and flow-metabolic coupling and uncoupling in preclinical models of ischemic conditions such as CA and stroke.

It is important to note that in this study, we observed large variation among the absolute CMRO$_2$ values for the individual animals; this may be attributed to the lengthy experimental procedures performed prior to the measurements. For these animals, several hours of surgery took place on the day of the experiment to implant electrophysiology leads, intubate and cannulate the animals, and perform the partial craniectomy for optical imaging. These procedures likely contributed to stress in the animals that could have resulted in significant variation in cerebral metabolic state between the animals at the time the measurements were performed. To address this issue, future studies can reduce stress on the animals by (1) employing a thinned-skull technique instead of a craniectomy and (2) implanting electrophysiology leads on a day prior to the experiment.

4.1 Optical Imaging Segments Venous Regions to Better Quantify Cerebral Oxygen Extraction

The imaging capability of our device allows the segmentation of an ROI atop a prominent vein, which enables more accurate measurements of the quantity of deoxygenated venous blood and, hence, the quantity of oxygen consumed by the brain. With the use of a larger ROI, the local CMRO$_2$ would be systematically underestimated due to inclusion of the parenchyma in the ROI, as oxygen extraction in the parenchyma is lower than in individual vessels. CMRO$_2$ models of diffuse light transport implicitly assume that the concentration of deoxygenated hemoglobin is that within the veins specifically and not the bulk tissue. However, most diffuse optics measurements of CMRO$_2$ are unable to satisfy this condition, as they typically use fiber-based spectroscopic techniques that sample the bulk tissue and thus cannot distinguish between venous and mixed arterial-venous parenchymal regions. In this report, the use of DOI allows us to obtain a spatial map of the tissue properties, enabling use of deoxyhemoglobin concentrations measured in a venous ROI to obtain more accurate quantitative values of CMRO$_2$.

4.2 Correction of CMRO$_2$ Data for Partial-Volume Effects

Due to the heterogeneity of biological tissues, partial-volume effects are a well-known confounding factor, especially with optical property mapping using a planar wide-field imaging technique such as SFDI. In our work, we require knowledge of Hb$_v$ [Eq. (6)]. However, due to partial volume effects, simple selection of an ROI that is coincident with a venule is insufficient. To address this issue, we employed a partial-volume correction to the CMRO$_2$ equation. To accurately incorporate this scaling term, it is necessary to know the concentration of total hemoglobin (Hb$_b$) within the blood of each animal. In this report, these values were acquired via ABG measurement before CA. A coefficient of variation of 13% in Hb$_b$ was determined from the measurements. If the variation in Hb$_b$ among the different subjects was not considered, an additional error of $\sim$12% to 25% in the measured CMRO$_2$ would be achieved due to this within-group variability in Hb$_b$ values.

4.3 Contributions of Directed Flow Versus Diffuse Flow

With diffuse optical measurements of blood flow, the model of blood flow typically is assumed to be either diffusion-like (i.e., Brownian motion) or directional (i.e., intravascular). Here, we assumed that the corrected flow speed was the latter [Eq. (2)]. Previous studies have used the diffusion-like term as the free parameter when fitting for flow speed or constrained the fit in a model system such that one could choose to fit for either diffuse or directed flow but not both simultaneously. Recently, Postnov et al. used high-speed LSI to map the autocorrelation
function pixel-by-pixel in the rodent brain, identifying the dominant type of particle motion at each pixel. They observed that the directed flow term was dominant in large vessels, whereas the diffuse flow term was dominant in the parenchyma.

Here, we could not rigorously solve for the autocorrelation function because the sampling frequency of our LSI data acquisition was too low to perform a method similar to that of Postnov et al. Instead, we used a two-step approach of (1) using SFDI data to account for the effects of optical properties on interpreting the LSI data and (2) fitting the resulting corrected data to a model of directed flow to extract a characteristic flow speed. This method provided characteristic flow speeds that were similar to previously reported values.

4.4 Limitations of Zero-Flow Condition

Our current approach for measuring absolute CMRO$_2$ requires temporary induction of a “zero-flow” condition in the brain. In this report, this condition was met using a CA model in rats. However, there is a clear need to investigate alternative approaches for interrogating absolute CMRO$_2$ without creating harmful perturbations. Future work can incorporate techniques such as balloon occlusion tests, as sometimes done in the clinical setting, to temporarily induce a zero-flow condition that can be quickly reversed without long-term harm to the animal. However, these procedures may have longer-term effects on the brain, so they would require evaluation within our preclinical model. Although the comparison of absolute CMRO$_2$ calculated with our approach with PET and MRI is encouraging, further comparison work is required with measurements collected from the same animals under identical anesthesia conditions. Future work in this area is warranted. Additional future studies will assess the sensitivity of our CMRO$_2$ method to small perturbations in brain metabolism over time in longitudinal studies. This future investigation will involve comparison of our method with an established modality such as MRI in preclinical chronic imaging experiments.

5 Conclusion

Here, we have described, to the best of our knowledge, the first report of absolute CMRO$_2$ mapping in the rat brain using DOI. Absolute CMRO$_2$ allows for quantitative assessment of cerebral metabolism without the need for baseline measurements, enabling longitudinal comparison between animals and among multiple days of measurement on an absolute rather than relative scale. The CMRO$_2$ measurements provided by our multimodal system were in good agreement with those previously measured in the brain of anesthetized rats using PET and MRI. This method shows significant potential for assessing and monitoring cerebral metabolism and predicting cerebral response to ischemic injury.

Disclosures

B. J. T. is a cofounder of Modulim and has no financial interest. The other authors have no competing financial interests to discuss.

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Code, Data, and Materials Availability

Custom MATLAB code used in this report is available upon request by contacting Prof. Robert Wilson (wilsonrh@uci.edu).

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Biographies of the authors are not available.