Estimation of Cortical Oxygen Metabolism in Awake Mice using Two-photon Imaging of *Oxyphor 2P*

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Abstract: With the recently improved oxygen sensor *Oxyphor 2P*, we performed two-photon phosphorescence lifetime imaging of partial pressure of oxygen gradients surrounding diving arterioles in awake mice and estimated oxygen metabolism using the Laplace method.

1. Introduction

The brain is continuously supplied with oxygen, which is metabolized in mitochondria to provide the energetic substrates needed to maintain the activity of nerve cells. Because the oxygen concentration is spatially variable in healthy and diseased brain, a method that can measure the partial pressure of oxygen (pO_2) with microscopic resolution would be favorable. This recently became possible due to development of O_2 probes for 2-photon Phosphorescence Lifetime imaging (2PLM). We used 2PLM to measure pO_2 in the brain of awake mice using the recently improved pO_2 sensor *Oxyphor 2P* [2]. We injected the pO_2 sensor in the vicinity of a diving arteriole in the mouse cortex and measured pO_2 as a function of the radial distance from the arteriole and at different cortical depths below the pial surface. Here we apply the Laplace method to these data for estimation of cerebral rate of O_2 consumption ($CMRO_2$).

2. Methods

Animal Procedures: Mice were implanted with chronic "cranial windows" over the somatosensory cortex, providing a clear optical access to the underlying cortical tissue. The windows contained a port filled with silicone, so ~ 4 weeks after surgery the pO_2 sensor could be injected through the port in the vicinity of a diving arteriole. We were able to perform repeated injections of the sensor and longitudinal measurements of several arteries per mouse.
Imaging: Imaging was performed using an Ultima 2-photon laser scanning microscope integrated with a time-correlated single photon counting card (SPC-150, Becker & Hickl). *Oxyphor 2P* was excited at 950 nm using a Coherent Ultra II laser. We used a custom-made filter set to collect phosphorescence at ca. 730 nm using a Gallium arsenide photomultiplier (H7422P-50, Hamamatsu). Phosphorescence was collected in 300 μs cycles starting with a 13 μs excitation gate from a square or radial grid of 20x20 points covering 200x200 μm centered on a diving arteriole. We collected 50 cycles per point (50x300 μs = 15 ms). The entire grid of 400 points was collected in 2 min. We recorded mouse movements with an accelerometer and used this data to exclude periods with excessive motion. Phosphorescent decay histograms for each point were summed over the repetitions to reach >500 cycles/point before fitting decays. Custom-written MATLAB code was used to estimate pO_2 in [mmHg] using Stern-Volmer calibration plots. Simulation: A geometry including a central arteriole and a peripheral arteriole (each of 50 mmHg periarteriolar pO_2 and 7.5 μm radius) was simulated and pO_2 values synthesized for a radial grid analogous to the in vivo measurements: A constant $CMRO_2$ of 1 μmol/cm^3/min and a no-flux boundary condition at 120 μm radius were set. The Poisson equation that relates steady-state oxygen diffusion gradients to $CMRO_2$ was solved numerically given certain boundary conditions.

3. Results

Previously, we used periarteriolar pO2 gradients to estimate $CMRO_2$ using the Krogh model [3]. This model requires an accurate estimation of the parameter Rt, which is defined as a no-flux boundary condition. Because an error in estimation of Rt leads to an error in estimation of $CMRO_2$, we tested an alternative approach based on the diffusion

of oxygen as formulated by the Laplace operator (a double spatial derivative) applied on smoothed radial pO_2 profiles. For this purpose, we interpolated radial pO_2 values in 20 radial directions and applied smoothing along each direction using a cubic spline function in Matlab (*csaps*). Then, we calculated the second spatial derivative along each of the radial directions. This method was validated using synthetic pO_2 data based on a numerical solution of the diffusion equation for a given "ground truth" $CMRO_2$.

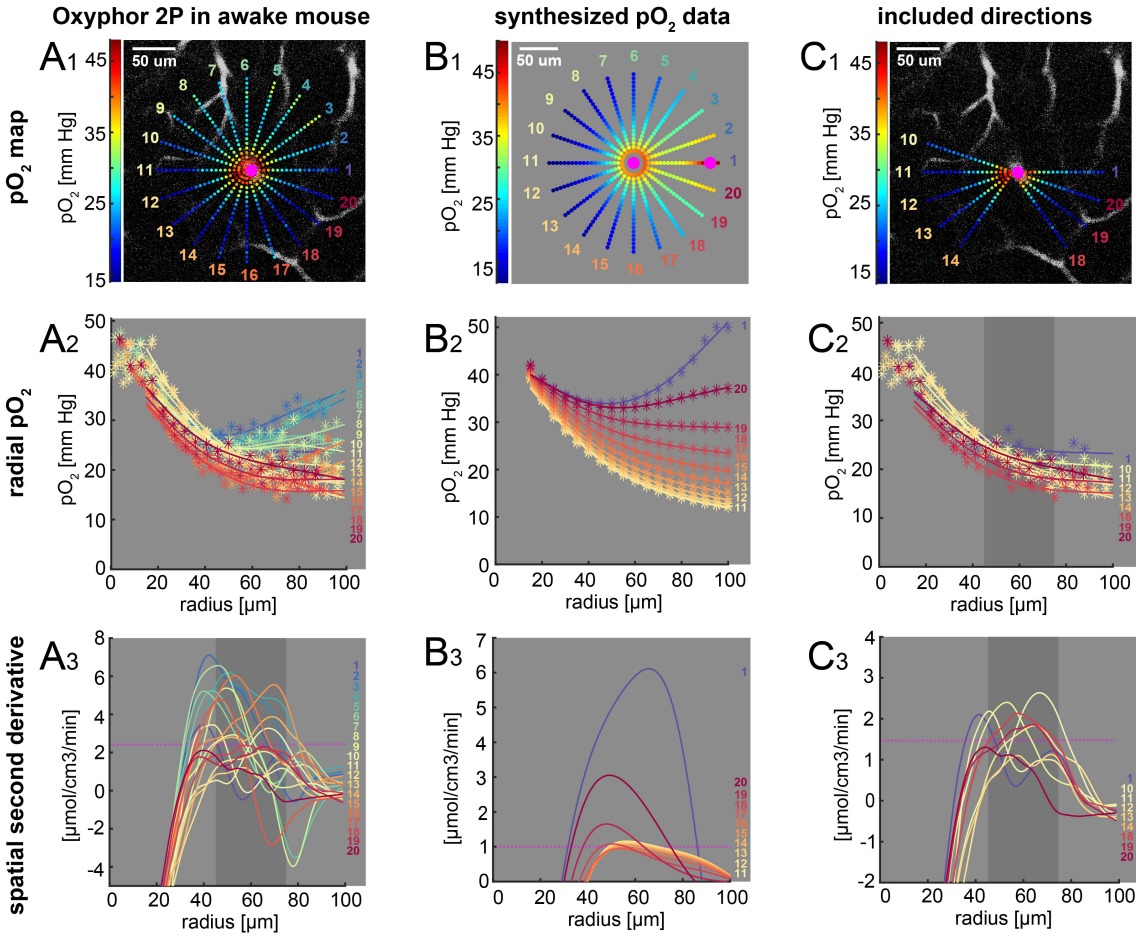

Figure1: Extracting $CMRO_2$ from periarteriolar pO_2 gradients using the Laplace method. A) The radial grid of pO_2 measurements was superimposed on the vascular reference images (A_1). Along each radial direction, the cubic smoothing spline interpolation (A_2), and estimation of $CMRO_2$ using the spatial second derivative (A_3) was applied. B) Simulation: the effect of a second oxygen source is demonstrated with synthesized pO_2 data with the true $CMRO_2$ of 1 µmol/cm^3/min. C) To avoid bias due to additional oxygen sources from in vivo data, radial directions with increasing pO_2 values between 45 and 75 µm were excluded.

4. Conclusion

Our new approach based on the Laplace operator applied on smoothed radial pO_2 profiles provides a viable alternative to the Krogh model when the parameter Rt cannot be accurately estimated. This method will allow a future testing of specific biological hypotheses such as the common notion of higher $CMRO_2$ in cortical layer IV compared to more superficial layers.

5. Acknowledgements

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6. References

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