Direct and simultaneous 2-photon imaging of tissue and intravascular oxygenation during the response to sensory stimulation *in vivo*

Anna Devor[1,2,3], Sava Sakadžić[1], Payam A. Saisan[3], Mohammad A. Yaseen[1], Emmanuel Roussakis[4], Peifang Tian[3], Vivek J. Srinivasan[1], Anders M. Dale[2,3], Sergei A. Vinogradov[4], David A. Boas[1]

[1]Martinos Center for Biomedical Imaging, MGH, Harvard Medical School; [2]Departments of Neurosciences and [3]Radiology, UCSD; [4] Department of Biochemistry and Biophysics, University of Pennsylvania

Summary

Brain activity largely relies on mitochondrial oxidative metabolism to meet the energy demands. However, our ability to probe microscopic availability of O_2 during different levels of neuronal activity has been limited to point measurements using O_2 electrodes. To overcome this limitation, we applied a novel phosphorescent probe for simultaneous 2-photon imaging of tissue and intravascular pO_2 in the rat primary somatosensory cortex.

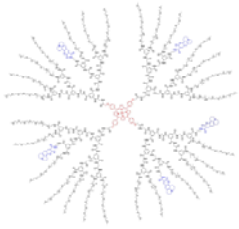
We measured pO_2 gradients at different cortical depths (down to 250 μm) and distance to the closest arteriole or venule. We observed steep gradients of pO_2 around the diving arteries and lesser gradients around most of the surfacing venules. In high-resolution scans, pO_2 gradients were also observed around some capillaries. Sensory stimulation induced an increase in tissue and intravascular pO_2 that was mostly pronounced in tissue away from the feeding arterioles and around surfacing venules. In some instances, this response was preceded by an initial decrease in pO_2 (the "initial dip"). Temporal characteristics and amplitude of this "functional" pO_2 signal change were determined by proximity to arterioles or venules, and the baseline pO_2 level.

Methods

Animals: Rats were anesthetized with α-chloralose. HR, BP, expired CO_2 and temperature were continuously monitored. Respiration was aimed to maintain pCO_2 between 35 and 45 mmHg. Stimulation consisted of a train of 100-μs electrical pulses delivered to a forepaw at 3 Hz for duration of 2 s.

Cortical exposure: An area of skull overlying SI was exposed, the skull and dura mater were removed, and the space between the exposed brain surface and the coverglass was filled with ~1 % (w/v) agarose (Sigma) in ACSF.

Dyes: After mapping the neuronal response using surface potentials, phosphorescent pO_2 indicator PtP-C343 (Figure 1) was dissolved in ACSF and microinjected in the center of the responsive area. SR101 was applied to the surface. The same pO_2 indicator was injected IV. To visualize the vasculature, ~5 % (w/v) solution of fluorescein- or rhodamin-conjugated dextran in saline was injected IV.



Imaging: Images were obtained using a home-built 2-photon microscope. We used a 4x objective (Olympus XLFluor4x/340, NA=0.28) to obtain images of the surface vasculature across the entire cranial window to aid in navigating and a 20x water-immersion objective (Olympus, XLUMPlanFI20x NA=0.95) for high-resolution and functional imaging. Data analysis was performed in Matlab.

Figure 1. Structure of the probe PtP-C343 peripherally modified with polyethyleneglycol amine residues (Av. MW 2000; n=37-38).

Baseline pO_2 landscape reflects vascular geometry

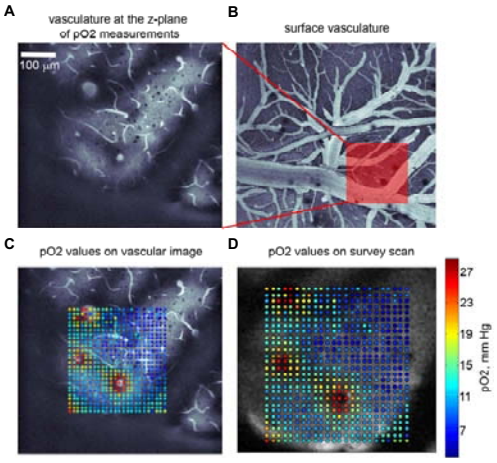


Figure 2. Baseline pO_2 is elevated around diving arterioles and surfacing venules.

A. A vascular image around the depth plane of pO_2 measurements (80 μm). The image was calculated as a maximum intensity projection (MIP) of a 16-μm image stack; individual images were acquired every 2 μm.

B. An image of the surface vasculature; the red square indicates the XY position of A.

C. The MIP image from A with superimposed baseline pO_2 values.

D. The same pO_2 values superimposed on the corresponding image of porphyrin phosphorescence ("survey scan").

Sensory stimulation causes an increase in pO_2

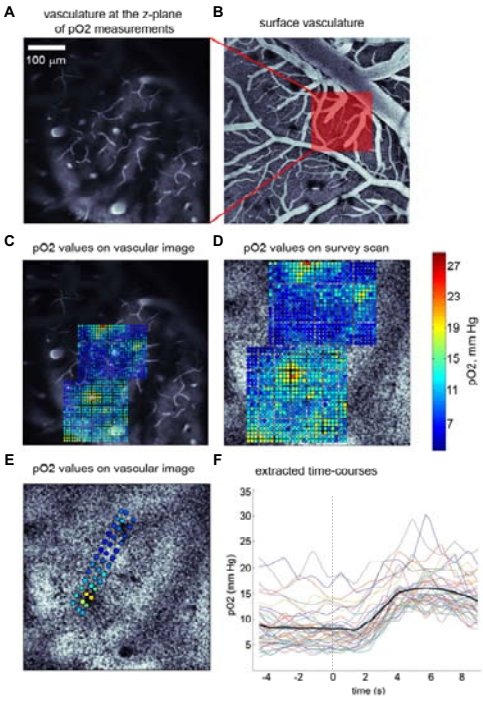


Figure 3. Functional pO_2 imaging.

A. A vascular image around the depth plane of pO_2 measurements (120 μm). The image was calculated as a maximum intensity projection (MIP) of a 16-μm image stack; individual images were acquired every 2 μm.

B. An image of the surface vasculature; the red square indicates the XY position of A.

C. The MIP image from A with superimposed baseline pO_2 values.

D. The same pO_2 values superimposed on the corresponding survey scan .

E. Points used for measurement of time-courses of pO_2 change in response to stimulation. The points are colored according to their baseline pO_2 value.

F. Time-courses of pO_2 change extracted from the points in E. Each curve represents an average of 10 stimulus trials. 2000 phosphorescent decays were averaged for every time point.

A trend for an initial decrease is observed in tissue away from feeding blood vessels

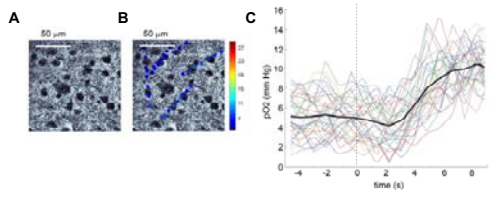


Figure 5. High-resolution functional imaging of tissue pO_2

A. A high-resolution survey scan 175 μm below the cortical surface.

B. The same survey scan with superimposed color-coded baseline pO_2 values. The colorbar in mm Hg is shown on the right.

C. Time-courses of pO_2 change extracted from each point shown in B. The thick black line represents an average across all measured points. The dashed line indicates stimulus onset.

The temporal profile and magnitude of tissue pO_2 increase during the hemodynamic response varies as a function of the baseline pO_2

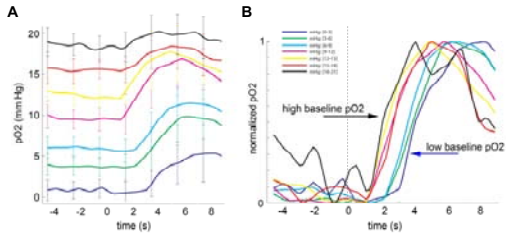


Figure 4. The onset time, time-to-peak and magnitude of pO_2 change in response to a sensory stimulus depend on the baseline pO_2 value.

A. Averaged time-courses of pO_2 signal change, grouped according to the baseline (prestimulus) pO_2. The dashed line indicates stimulus onset. About 100 measurement points from 4 subjects were averaged for each category.

B. The same time-courses as in A, normalized to the peak value. The dashed line indicates stimulus onset.

Geometrical position relative to the feeding vessels underlies the dependence of functional responses on the baseline pO_2

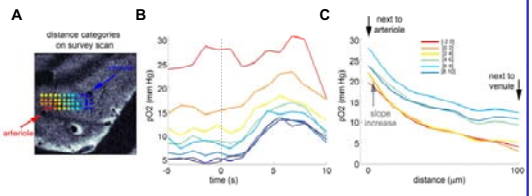


Figure 6. Functional pO_2 changes in XY plane along the axis between neighboring diving arteriole and surfacing venule.

A. A survey scan with superimposed color-coded baseline pO_2 values 80 μm below the cortical surface. A grid of baseline pO_2 values for the same location is shown in Figure 2.

B. Averaged time-courses of pO_2 signal change, grouped according to the baseline (prestimulus) pO_2. The groups are color-coded in A and B. The dashed line indicates stimulus onset.

C. pO_2 profiles between the arteriole and venule. Profiles extracted from different times relative to the stimulus onset are superimposed. The inset shows the color code (in s relative to the stimulus onset).

Conclusions

- ***The phosphorescent pO_2 indicator PtP-C343 can be used for simultaneous imaging of tissue and intravascular pO_2 at baseline and in response to a brief (2s) sensory stimulus.***
- ***Baseline pO_2 values reflect the surrounding vascular geometry. The pO_2 landscape is dominated by large gradients around diving arterioles. Gradients are also observed around surfacing venules and some capillaries.***
- ***The onset of functional pO_2 response varies as a function of baseline pO_2. The earliest increase in pO_2 occurs at locations with the highest baseline pO_2 level around arterioles. The most delayed increase occurs at locations with the lowest baseline pO_2 level in the tissue away from vessels (in between capillaries). These onset differences are probably caused by a combination of two factors: vascular transit times and O_2 diffusion.***
- ***A trend for an initial decrease in pO_2 is observed in tissue remote from feeding vessels.***
- ***The slope of spatial pO_2 gradient around diving arterioles becomes steeper during the rising phase of the response. Later in the response the slope returns to the baseline. This behavior is consistent with an initial increase in blood oxygenation limited to arterioles followed by that in the capillary bed.***
- ***During an increase in blood oxygenation in response to the 2-s stimulus the tissue pO_2 does not drop below the baseline.***

Acknowledgements

We gratefully acknowledge support from the NINDS (NS051188, NS057198, NS055104, NS057476) and NIBIB (EB009118, EB007279, EB000790)