Journal of Neuroscience Methods 281 (2017) 1–6

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Research article

Planar implantable sensor for in vivo measurement of cellular oxygen metabolism in brain tissue

Vassiliy Tsytsarev[a,*], Fatih Akkenti[a], Elena Pumbo[b], Qinggong Tang[c], Yu Chen[c], Reha S. Erzurumlu[a], Dmitri B. Papkovsky[d,*]

[a] Department of Anatomy and Neurobiology, University of Maryland School of Medicine, 20 Penn Street, HSF-2, 21201 MD, Baltimore, USA
[b] Center for Genetic Medicine, Children's National Medical Center, Washington DC, 111 Michigan Avenue, NW Washington, DC 20010, USA
[c] Fischell Department of Bioengineering, University of Maryland, College Park, Kim Engineering Building, College Park, MD 20740, USA
[d] School of Biochemistry and Cell Biology, University College Cork, Cavanagh Pharmacy Building 1.28, College Road, Cork, Ireland

HIGHLIGHTS

- We visualized local oxygenation in the brain using an oxygen sensor comprising a polymeric film with a phosphorescent oxygen-sensitive coating.
- We demonstrate the possibility of dynamic mapping of relative changes in oxygen concentration in live mouse brain tissue with such sensor.
- Our results provide evidence that our sensor can be used as a tool in visualization and analysis of sensory-evoked neural activity in vivo.

ARTICLE INFO

Article history:
Received 31 October 2016
Received in revised form 13 January 2017
Accepted 15 February 2017
Available online 20 February 2017

Keywords:
Barrel field
In vivo optical imaging
Functional brain mapping
Quenched-phosphorescence oxygen sensing
Planar oxygen sensor
Somatosensory cortex
Whisker stimulation

ABSTRACT

Background: Brain imaging methods are continually improving. Imaging of the cerebral cortex is widely used in both animal experiments and charting human brain function in health and disease. Among the animal models, the rodent cerebral cortex has been widely used because of patterned neural representation of the whiskers on the snout and relative ease of activating cortical tissue with whisker stimulation.
New method: We tested a new planar solid-state oxygen sensor comprising a polymeric film with a phosphorescent oxygen-sensitive coating on the working side, to monitor dynamics of oxygen metabolism in the cerebral cortex following sensory stimulation.
Results: Sensory stimulation led to changes in oxygenation and deoxygenation processes of activated areas in the barrel cortex. We demonstrate the possibility of dynamic mapping of relative changes in oxygenation in live mouse brain tissue with such a sensor.
Comparison with existing method: Oxygenation-based functional magnetic resonance imaging (fMRI) is very effective method for functional brain mapping but have high costs and limited spatial resolution. Optical imaging of intrinsic signal (IOS) does not provide the required sensitivity, and voltage-sensitive dye optical imaging (VSDi) has limited applicability due to significant toxicity of the voltage-sensitive dye. Our planar solid-state oxygen sensor imaging approach circumvents these limitations, providing a simple optical contrast agent with low toxicity and rapid application.
Conclusions: The planar solid-state oxygen sensor described here can be used as a tool in visualization and real-time analysis of sensory-evoked neural activity in vivo. Further, this approach allows visualization of local neural activity with high temporal and spatial resolution.

1. Introduction

Localization and real-time monitoring of spontaneous and evoked neuronal activity in the brain are important in understanding the functional characteristics of neural networks and brain function (Bouchard et al., 2009; Inyushin et al., 2001; Lenkov et al., 2013; Silasi et al., 2016; Zhang et al., 2014).

* Corresponding authors.
E-mail addresses: tsytsarev@som.umaryland.edu, tsytsarev@seas.wustl.edu (V. Tsytsarev), akkentli@umaryland.edu (F. Akkenti), EPumbo@childrensnational.org (E. Pumbo), qtang@umd.edu (Q. Tang), yuchen@umd.edu (Y. Chen), RErzurumlu@som.umaryland.edu (R.S. Erzurumlu), d.papkovsky@ucc.ie (D.B. Papkovsky).

http://dx.doi.org/10.1016/j.jneumeth.2017.02.005
0165-0270/

Over the last two decades a number of optical methods have been developed for functional brain imaging. Most of them are based on the detection of changes in blood flow, tissue oxygenation and energy usage (Lenkov et al., 2013; Liao et al., 2013). VSDi method was used in several studies, including novel 3D visualization of the neural activity, however due to cytotoxicity of the indicator dye it cannot be used in clinical practice (Tang et al., 2016). Changes in local oxygenation and glucose consumption induced by changes in neural activity Tsytsarev et al., 2013) allow indirect monitoring of neural activity with these metabolic markers. Two-photon enhanced phosphorescent nanoparticles in combination with two-photon in vivo imaging have been successfully used for monitoring of the oxygen partial pressure in the cortical tissue in vivo (Sakadzic et al., 2010, 2011). It is also possible to use single photon excitation using phosphorescence oxygen sensitive dye, for example Oxyphor R3 (Sakadzic et al., 2011). Each method has its advantages and limitations, but only a few can be used in long-term experiments and in clinical settings. This motivated us to develop a new approach, which overcomes toxicity side effects of sensor dyes and which is better suited for functional brain mapping applications.

The rodent whisker neural system (which consists of a patterned array of whisker follicles on the snout, the trigeminal ganglion neurons that innervate them, whisker-specific neural modules, the barrelettes in the brain stem, the barreloids in the thalamus, and the barrels in the primary somatosensory cortex) is a useful model for studying peripherally evoked neural activity in the central nervous system (Erzurumlu and Gaspar, 2012). Mechanical stimulation of a single whisker evokes neural activity all along the system and ultimately in the corresponding barrel of the contralateral hemisphere. Barrels are located in cortical layer IV, so they are accessible for optical monitoring of whisker-evoked neural activity in vivo, through a cranial window (Tsytsarev et al., 2013).

Optical imaging methods based on the monitoring of local brain oxygenation are useful tools for studying different cortical regions and physiological processes in these regions (Chen et al., 2003). Activity patterns obtained in our experiments are similar to the blood oxygenation level dependent signals (BOLD) produced in fMRI or activity patterns obtained by near-infrared spectroscopy (NIRS) (Fukuda et al., 2016; Lenkov et al., 2013). Both BOLD fMRI and NIRS methods employ hemoglobin as an endogenous contrast agent and visualize neuronal activity indirectly via correlations with local metabolism (Arthurs and Boniface, 2002). In contrast, the use of a planar solid-state oxygen sensor together with phosphorescence imaging described here is direct and measures the concentration of free oxygen dissolved in the tissue.

Fluorescence based imaging, using different probes and contrast agents, has grown considerably in recent years (Baker et al., 2015; Jaafari et al., 2015; Mutoh et al., 2015; Tsytsarev et al., 2012; Liao et al., 2013). VSDi allows direct monitoring of excitatory neural activity (Tsytsarev et al., 2012), while IOS (Inyushin et al., 2001) reflects optical changes in cortical tissue associated with differential absorption of light by oxy- and deoxyhemoglobin (Hu et al., 2009; Kalchenko et al., 2014, 2015).

In this study, we describe the use of a planar solid-state oxygen sensor comprising a polymeric film with a phosphorescent oxygen-sensitive coating on the working side. This sensor, when applied topically on mouse dura mater surface, allows dynamic real-time in vivo imaging on a commercial fluorescent imager of deoxygenation and reoxygenation in specific areas of cortical tissue, with high spatial and temporal resolution. Obtained data demonstrate that the planar sensor provides simple and efficient means for the dynamic 2D imaging of the oxygen levels in cortical tissue in vivo .

2. Materials and methods

The planar oxygen sensor was produced by applying a polymeric cocktail of PtBP dye (Luxcel Biosciences) on the surface of polyester film, Mylar® (Du Pont, 22 × 22 mm pieces) using a 6800 Spin Coater machine (SCS) at 3000 rpm (0.2 ml per sample). The coated films were then cut into 5 × 5 mm pieces of planar oxygen sensors, which were used in the animal experiments (Fig 1A). Planar sensor, which is shown at the Fig. 1B, was also tested in Ringer solution alone, which is similar in composition to the vertebrate body fluids. The fluctuation of fluorescence signal did not exceed 0.05 ΔF/F(%) (Fig. 1C).

The in vivo imaging experiments were performed in six male and female mice (B6, weight 20–35 g, aged 3–6 months) using a MiCAM-02 optical imaging system (SciMedia, Ltd., Fig. 1A). The subjects used here were C57BL/6N (B6) line, which was originally obtained from Charles River Laboratory (Frederick, MD) and maintained in the University of Maryland School of Medicine animal facility. All animal handling was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996 and a protocol approved by the University of Maryland School of Medicine Institutional Animal Use and Care Committee.

Animals were anesthetized with an intraperitoneal injection of urethane (1.15 g/kg).

The anesthetized mouse was fixed on a stereotaxic frame, the skin and muscles of the dorsal part of the head were removed surgically, and an approximately 10 mm^2 part of the skull above the left parietal cortex was removed using a dental drill. The exposed area was washed with a hemostatic sponge with the artificial cerebrospinal fluid (ACSF).

A planar sensor was applied to the exposed cortex, with the oxygen-sensitive surface facing down to dura mater surface in immersion contact with the tissue. In the imaging session the CCD camera was positioned above the recording area and directed such that its optical axis was perpendicular to the area of recording. The sensor was excited with 630 ± 10 nm light and emission (maximum at 760 nm) were recorded with a 650 nm long-pass filter. At the start of each imaging session a grayscale image of the monitored area was captured and saved as a graphic file. The imaged session consisted of 100 trials with 200 frames per trial measured at 50 ms per frame and inter-trial intervals of 30s.

Mechanical stimulation of the E2 whisker was delivered by specially designed electromechanical stimulator at the 50th frame. Each stimulation consisted of ten 25-ms deflections with a 25-ms inter-pulse interval.

Phosphorescent signals in the recording area were analyzed using the Brain Vision Analyzer (Brain Vision Inc., Tokyo, Japan) and presented as ΔF/F (%). One of the limiting factors in functional brain mapping is low-frequency biological noise (<0.3 Hz (Grinvald et al., 1999)). When signal measurement time is significantly longer (10 s in our case), biological noise is present in all of the imaging frames acquired during that trial. However, the first frame taken prior to the whisker stimulation, contains only this biological noise and no other components that relate to stimulus elicited activity. Therefore, subtraction of the first frame from all subsequent frames can significantly reduce the overall noise and improve data analysis (Grinvald et al., 1999). As a result of this procedure, phosphorescence response at each time point is presented as Δ F/F (%) for the whole area of imaging, where F is mean pixel intensity in this area. This is a common way to analyze quite noisy intrinsic (Grinvald et al., 1999) and extrinsic (Grinvald and Hildesheim, 2004) brain optical imaging data.

After the measurement session animal was euthanized by overdose of urethane. Immediately after this sensor phosphorescence