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Development of new optical imaging systems of oxygen metabolism and simultaneous measurement in hemodynamic changes using awake mice

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HIGHLIGHTS

- New optical imaging system for oxygen metabolism and hemodynamic changes.
- Simultaneous measurement of LSI and IOSI performed on mouse brain in awake state.
- Simultaneous measurement of LSI and IOSI was verified by laser Doppler flowmetry.
- FAI was performed from the same brain area as LSI and IOSI measurements.
- Results of new optical imaging system were in good agreement with human PET studies.

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ABSTRACT

Background: PET allows the measurement of CBF, CBV and $CMRO_2$ in human and plays an important role in the diagnosis of pathologic conditions and clinical research. On the other hand, in animal studies, there is no optical imaging system for evaluating changes in CBF and CBV, and oxygen metabolism, from the same brain area under awake condition.
New method: In the present study, we developed a simultaneous measurement system of LSI and IOSI, which was verified by LDF. Moreover, to evaluate oxygen metabolism, FAI was performed from the same brain area as LSI and IOSI measurements.
Results: The change in CBF according to LSI was correlated with that by LDF. Similarly, the change in CBV obtained by IOSI was also correlated with RBC concentration change measured by LDF. The change in oxygen metabolism by FAI was associated with that in CBF obtained by LSI, although the change in CBF was greater than that in oxygen metabolism.
Comparison with existing method(s): We revealed that the relationship between oxygen metabolism and CBF as measured by our system was in good agreement with the relationship between $CMRO_2$ and CBF in human PET studies.
Conclusions: Our measurement system of CBF, CBV and oxygen metabolism is not only useful for studying neurovascular coupling, but also easily corroborates human PET studies.

Abbreviations: PET, positron-emission tomography; CBF, cerebral blood flow; CBV, cerebral blood volume; $CMRO_2$, cerebral metabolic rate of oxygen; LSI, laser speckle imaging; IOSI, intrinsic optical signal imaging; LDF, laser Doppler flowmetry; FAI, flavoprotein autofluorescence imaging.

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1. Introduction

In order to understand the mechanism of neurovascular coupling, hemodynamic response associated with neural activity was investigated using positron-emission tomography (PET) and functional magnetic resonance imaging (fMRI) in human. Neural activation has been reported to cause an increase in the cerebral metabolic rate of oxygen ($CMRO_2$) and a larger increase in cerebral blood flow (CBF) than in cerebral blood volume (CBV) in human PET studies (Ito et al., 2005). Crossed cerebellar diaschisis, which is caused by contralateral supratentorial lesions, has shown reductions in CBF and $CMRO_2$ in human (Lenzi et al., 1982; Yamauchi et al., 1992a,b, 1999a,b; Ito et al., 2002). Ito et al. (2002) reported that crossed cerebellar diaschisis can be considered as neural deactivation and that deactivation causes almost the same degree of decrease in CBV and CBF.

Animal studies, in which invasive procedures can be applied, can also be useful for investigating the mechanism of neurovascular coupling. It is well known that anesthesia greatly affects the animal physiological condition, including neurovascular coupling (Masamoto and Kanno, 2012). Especially, isoflurane anesthesia attenuates neural activity (Ferezou et al., 2006) and CBF response to sensory stimulation (Takuwa et al., 2012). Therefore, an awake animal model is essential for an accurate evaluation of neurovascular coupling. Recently, we developed animal experiment models of neural activation (Takuwa et al., 2011, 2012) and neural deactivation (Takuwa et al., 2013b) using awake mice. The animal models showed a greater increase in CBF than in red blood cell (RBC) concentration during neural activation, and almost the same degree of decrease in CBF and RBC concentration during neural deactivation, which was in good agreement with PET human studies (Ito et al., 2002, 2005). Although our above-mentioned method was an accurate and suitable approach for the investigation of hemodynamics, it lacks spatiotemporal resolution and oxygen metabolism measurements. To clarify the mechanism of brain function and brain disorders, a live imaging system for obtaining the hemodynamics and oxygen metabolism from the same animal brain is required.

In our previous study, we developed and validated a method for live imaging of CBF in awake mouse models using laser speckle imaging (LSI) (Takuwa et al., 2011). Other studies have used intrinsic optical signal imaging (IOSI) for the mapping of CBV (Bahar et al., 2006; Zhao et al., 2009; Martin et al., 2006). For a detailed investigation of the relationship between CBF and CBV in animal models, a method for the simultaneous measurement of CBF and CBV would be advantageous, in addition to measuring oxygen metabolism. In the present study, we aimed to develop a simultaneous measurement method of LSI and IOSI for the mapping CBF and CBV in awake mice and validate the results using laser Doppler flowmetry (LDF). We also performed flavoprotein autofluorescence imaging (FAI) from the same brain area as for the LSI and IOSI measurements. The autofluorescence of flavoproteins associates with the electron transport chain in mitochondria and provides a mapping of oxygen metabolism (Shibuki et al., 2003). Our measurement system of CBF and CBV and oxygen metabolism is not only useful for the study of neurovascular coupling using model animals, but it also readily corroborates human PET studies, which can measure CBF, CBV and $CMRO_2$.

2. Materials and methods

2.1. Animal preparation

A total of 8 male C57BL/6J mice (20–30 g, 7–11 weeks; Japan SLC, Inc., Hamamatsu) were used in three separate measurements: (1) simultaneous measurements of IOSI and LSI, (2) FAI measurements and (3) LDF measurements (Fig. 1A). These mice were housed with ad libitum food and water in their cages at 25 °C in a 12-h light/dark cycle. All experiments were performed in accordance with the institutional guidelines on humane care and use of laboratory animals and were approved by the Institutional Committee for Animal Experimentation.

The animals were anesthetized with a mixture of air, oxygen, and isoflurane (3–5% for induction and 2% for surgery) via a facemask, and a cranial window (3–4 mm in diameter) was attached over the left somatosensory cortex (including the somatosensory barrel cortex). A custom metal plate was affixed to the skull with a 7-mm-diameter hole centered over the cranial window. The method for preparing the chronic cranial window was previously reported in detail by Tomita et al. (2005) and Takuwa et al. (2013a, b). All experiments were performed two weeks after the cranial window surgery.

2.2. Experimental protocols

The experimental protocol for measurements using awake mice was reported previously (Takuwa et al., 2011, 2012, 2013a,b). Briefly, the metal plate on the animal's head was screwed to a custom-made stereotactic apparatus. The animal was then placed on a styrofoam ball that was floating using a stream of air. This allowed the animal to exercise freely on the ball while the animal's head was fixed to the apparatus. Under this condition, IOSI, LSI, FAI and LDF measurements were performed in the somatosensory cortex.

Hemodynamic response to neuronal activation was induced by whisker stimulation. An air puff was delivered to all of the right whiskers at a pressure of ~15 psi via a compressed-air bottle. Rectangular pulse stimulation (50-ms pulse width and 100-ms onset-to-onset interval, i.e., 10 Hz frequency) generated with a Master-8 (A.M.P.I., Jerusalem, Israel) was induced for a 10-s duration (Takuwa et al., 2011).

2.3. Simultaneous measurement of IOSI and LSI

We newly developed a custom setup for the simultaneous measurement of IOSI and LSI (Fig. 1B). The exposed cortical surface in the cranial window was simultaneously illuminated with a halogen light (wavelength: 570 nm) and a laser diode (wavelength: 780 nm). In IOSI measurements, a reduction rate of reflection at 570 nm, which is an isosbestic wavelength of hemoglobin, can allow the measurement of total hemoglobin and is closely correlated to CBV if hematocrit remains constant and proportional to CBF (Martin et al., 2006; Zhao et al., 2011; Ma et al., 2013). LSI with the laser diode at 780-nm wavelength provided the measurement of CBF (Konishi et al., 2002; Takuwa et al., 2011). The mean blur rate (MBR) can be calculated from a speckle pattern to map the CBF level. The LSI method was previously reported in detail by Takuwa et al. (2011). LSI and IOSI were simultaneously captured with two CCD cameras (MiCAM02, Brainvision, Tokyo, Japan) (left side of Fig. 1B).

Temporal resolution and spatial resolution were 50 Hz for 40 s (2000 frames/trial) and 192 × 128 pixels (each pixel size was 15 μm × 15 μm). Either 20 or 30 trials were repeatedly performed with an inter-trial interval of 30 s, and the image was averaged over the trials to improve the signal-to-noise ratio. IOSI was displayed as a percent change in illumination. Because a decrease in IOSI means an increase in CBV, we inverted the percentage change in IOSI to calculate the percentage change in CBV. The MBR of LSI was normalized to the baseline level (10-s pre-stimulus MBR values). The time–response curves of IOSI and LSI were recorded from a 1-mm diameter of the regions of interest (ROIs), which were displayed as black circles on the right side of Fig. 2. A picture showing