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Novel implantable imaging system for enabling simultaneous multiplanar and multipoint analysis for fluorescence potentiometry in the visual cortex

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ABSTRACT

Techniques for fast, noninvasive measurement of neuronal excitability within a broad area will be of major importance for analyzing and understanding neuronal networks and animal behavior in neuroscience field. In this research, a novel implantable imaging system for fluorescence potentiometry was developed using a complementary metal-oxide semiconductor (CMOS) technology, and its application to the analysis of cultured brain slices and the brain of a living mouse is described. A CMOS image sensor, small enough to be implanted into the brain, with light-emitting diodes and an absorbing filter was developed to enable real-time fluorescence imaging. The sensor, in conjunction with a voltage-sensitive dye, was certainly able to visualize the potential statuses of neurons and obtain physiological responses in both right and left visual cortex simultaneously by using multiple sensors for the first time. This accomplished multiplanar and multipoint measurement provides multidimensional information from different aspects. The light microsensors do not disturb the animal behavior. This implies that the imaging system can combine functional fluorescence imaging in the brain with behavioral experiments in a freely moving animal.

1. Introduction

A technique for real-time analysis of physiological neural activity within a freely moving animal would make a significant contribution to our understanding of neural networks and modification of behavior. In particular, a broad range and multipoint measurement, with enough spatiotemporal resolution for analyzing the differential propagation of neural activity between brain areas, is highly desired. In previous studies, positron emission tomography (PET), electroencephalography (EEG), and a small microscope of the head installation type have been developed for freely moving animals (Sossi and Ruth, 2005; Schulz et al., 2011; Ishida et al., 1993; Fan et al., 2011; Helmchen et al., 2001; Park et al., 2011). These compact instruments for functional brain measurements would contribute in revealing neural activities, which would provide insights into animal behavior in neuroscience. However, a new measurement technology is necessary for simultaneous clarification of chemical activity, dynamic anatomical character, and physiological processes with high spatiotemporal resolution in deep and wide area of the brain in conjunction with animal behavior.

Optical imaging is a useful and powerful tool because it enables wide range analysis with high spatiotemporal resolution without destroying the tissue. In particular, voltage-sensitive dye imaging, which describes a variation in a membrane potential as a change in fluorescence intensity, is able to analyze the activity of each neuron directly in real-time (Tasaki et al., 1968; Cohen et al., 1968, 1978; Davila et al., 1973; Salzberg et al., 1973; Grinvald et al., 1986; Grinvald and Hildesheim, 2004). A tiny implantable sensor for voltage-sensitive dye imaging fulfills these requirements.

CMOS (complementary metal-oxide semiconductor)-based multimodal microdevices can analyze various biological activities (Ng et al., 2006, 2008; Tamura et al., 2008; Ohta et al., 2009, 2011; Kobayashi et al., 2010a, Kobayashi et al., 2011; Sasagawa et al., 2011, 2012). A previous study demonstrated a novel on-chip imaging system that enabled potentiometric fluorescence imaging with primary cultured neurons (Kobayashi et al., 2010a). CMOS image sensor with multielectrode array for neural tissue was able to detect a local extracellular potential of the brain slice as a compact instruments

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(Nakajima et al., 2012), but the broad contact imaging with cellular resolution for the visualization of nerve activity at the tissue or the brain has not been achieved yet. The research reported here presents an innovative imaging system for fluorescence potentiometry using tiny implantable sensors. This proposed imaging system enables real-time fluorescence imaging in the brain. In addition, by using multiple sensors, multiplanar and multipoint measurements can be obtained simultaneously, providing multidimensional information from different aspects even in a complex structure.

2. Material and methods

2.1. Development of an implantable fluorescence imaging system

As shown in Fig. 1A, a novel imaging system was constructed according to a previous study (Kobayashi et al., 2010a) but with fresh technology and manifold modifications. The image sensor chip was designed (width, 1.0 mm; length, 3.5 mm) and was fabricated using a standard CMOS process (0.35 μm 2-poly-4-metal CMOS; Austria Microsystems) (Tagawa et al., 2010). The sensor had a light-sensing pixel array, and each pixel consisted of a three-transistor CMOS active pixel sensor (APS) (Nixon et al., 1996). The total number of pixels were 120 × 268, and each pixel size was 7.5 μm × 7.5 μm. A red filter (high-pass liquid photoresist filter; > 600 nm, FUJIFILM arch. Inc.) was superimposed over the pixel array and around the sensor chip to prevent the penetration of the excitation light from the LEDs. The 470–490 nm blue LEDs were used to efficiently excite a voltage-sensitive dye, RH795 (Molecular Probes, Inc.). The chip and blue or green LEDs (both blue (475 nm) and green (535 nm) bare LED chip, Epistar corp.) were assembled onto a polyimide substrate, which was designed to install LEDs around the sensor chip. The sensor was covered by a resin for waterproofing. An analog output data stream from the sensor was pre-amplified and converted to 12 bit digital data. The digitized data was processed by laboratory-made software and stored to a computer, and the image was reconstructed on a monitor. To produce the dual imaging system shown in Fig. 6, small transit boards that the mouse could carry on its back, twin compact control boards with an analog digital converter, and a coregulation software, were all developed and integrated. Image data were captured at a frame rate of < 60 fps.

2.2. Animal

B57BL/6J mice (SLC Co., Hamamatsu, Japan) were used in the present study. All animal procedures conformed to the animal care and experimentation guidelines of Nara Institute of Science and Technology.

2.3. Whole-brain slice culture

A urethane-anesthetized mouse brain was rapidly isolated and carefully maintained in an ice-cold Hanks' solution (Invitrogen, Inc.). The dura was removed and the isolated brain was embedded in a 4% low melting agarose gel (Nacalai Tesque, Inc.). The brain block was sectioned transversely using a razor and the slice including the visual cortex was prepared on the membrane filter as shown in Fig. 3B.

3. Results

3.1. Evaluation of fluorescence imaging by the newly developed imaging system

A novel real-time imaging system with multiple implantable microsensors for fluorescence potentiometry was developed

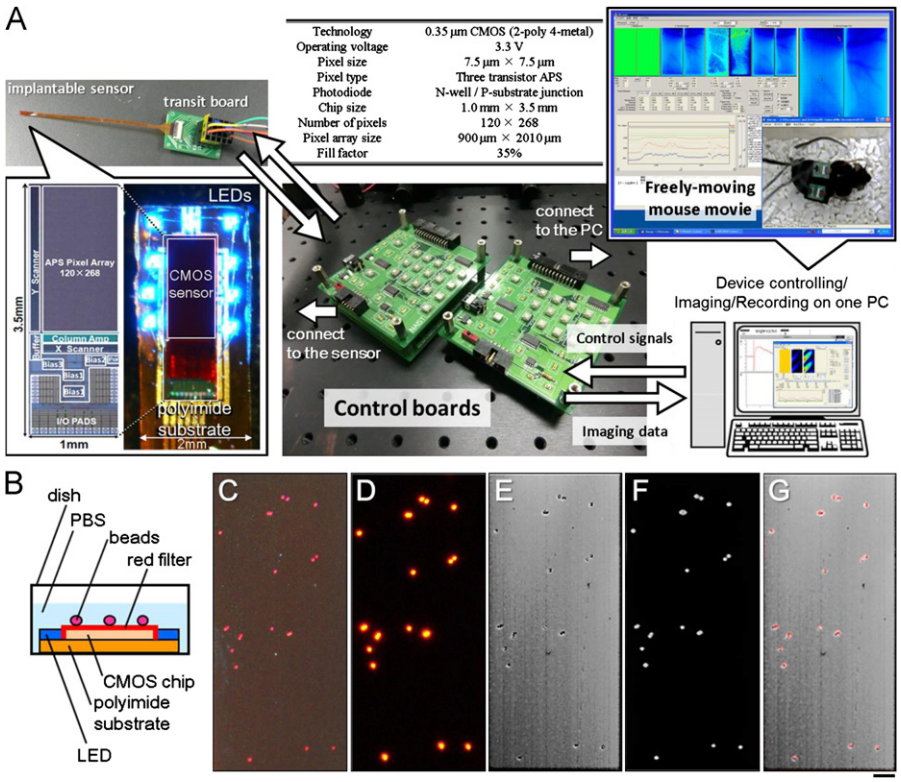

Fig. 1. Novel implantable fluorescence imaging system for a freely moving mouse. The fabricated imaging system is shown in (A). The information of the fluorescence image taken by the sensor was transferred to the transit board, to the control boards, and to the personal computer (PC). The fluorescent beads were placed on the sensor and were excited by the LEDs, as shown in (B). Bright field and fluorescence images were taken using a stereomicroscope (C, D). The bright field image using an external halogen light source (E) and the fluorescence image (F) were taken by the sensor. These images were then merged (G).