IMPLEMENTATION OF MULTI-FIBER PHOTOMETRY SYSTEM FOR EXPERIMENTAL SIMULATION OF FLUORESCENT TISSUE-PHANTOM AND DEEP BRAIN FLUORESCENT CALCIUM SIGNAL RECORDINGS IN VIVO

Yao-Wen Liang[1] , Han-Lin Wang[1], Chia Wei Liang[2] , You-Yin Chen[1]

[1]Department of Biomedical Engineering, National Yang Ming University, Taipei, Taiwan

[2]Department of Biomedical Engineering, Ming Chuan University, Taoyuan, Taiwan

INTRODUCTION

Calcium ions generate versatile intracellular signals that control vital functions in all the neurons. When an action potential occurred, the voltage-dependent Ca^{2+} channels open and make calcium ions flow intracellularly, and trigger exocytosis of neurotransmitter vesicles. Therefore, combined with Ca^{2+} fluorescent indicators for monitoring [Ca^{2+}] dynamics is a method for observing neural activity and has been widely used through numerous optical recording techniques so far.
Thus, we dedicated in developing a multi-fiber photometry system for recording neuronal activities in multiple brain areas simultaneously. First, we introduced computer simulation into finite element model to find the correlation between simulation and system with volume of tissue activated. Finally, the system was utilized to acquire *in vivo* fluorescent signals.

MATERAILS AND METHODS

Electrical Stimulation Model and Fluorescent Calcium Simulation. Neurons responds to the electrical stimulus and can be activated to generate action potential. Once the action potential travels down to the axonal terminal, the calcium ions flow intracellularly. By using the fluorescent calcium indicator, the calcium signals are converted to fluorescent signals and acquired by optical instruments. Therefore, to investigate the calcium signals during electrical stimulation, 3D model of the neural optrode was implemented and the finite element method (FEM) was used to estimate the potential distribution in brain tissue. The potential distribution (Ve) generated in the tissue medium was calculated using a frontal solution method from the Laplace equation：

$$\nabla 2Ve = 0$$

Surgery and Optrode Implantation. The optrode was made by an optical fiber (material: silicon dioxide) with 200 μm diameter, NA 0.48 and two microwires (material: stainless steel 304) with 50 μm diameter. The gap between optical fiber and microwire needs to be as close as possible. Position the optrodein place directly above the region of interest (AP: -3.48, ML: ±2.70, DV: 6.3) using the stereotaxic arm. The optrode should be advanced slowly at a rate of ~1 mm/min when inserting into the brain tissue. The ferrule of the optrode should rest on the remaining cranium. Subsequently, the whole skull was covered by the dental cement to strengthen the optrode connection with skull. Optrode could be released from holder when it was tightly fastened on skull and suture the scalp over the mound of dental cement. After implantation, rat was transferred under a heat lamp until wake up.
Simultaneous Electrical Stimulation and Fiber Optic Recording. Rats were placed on the stereotaxic apparatus, and the fluorescence signals were simultaneously recorded during electrical stimulation(**Fig.1)**. The stimulation consisted of 10 s for recording baseline fluorescence, on period of 20 s for electrical stimulation with different intensities of stimulation current (50 μA, 100 μA, 200 μA, 300 μA,), and 20s for resting.

RESUTLS AND DISCUSSION

VTAs with Different Stimulation Current Intensities. The VTA stimulated by the electrical stimulation combine with Monte Carlo simulation as shown in **Fig. 2**, where the blue part is the activated region in brain tissue. By using MC simulation, we simulated the excitation light distribution of an optical fiber (200 μm diameter, NA 0.48) in brain tissue. The light distribution could be used to determine whether the VTA could be illuminated by the excitation light and also the emission light in gray matter. The fluorescence intensity was collected by the same optical fiber with 200 μm diameter, 0.48 NA. As the current intensity increased, the activated volume increased and represented larger total calcium concentration; therefore, larger fluorescence intensity was detected consequently. The fluorescence intensity of 50 μA, 100 μA, 200 μA and 300 μA was 808.25 a.u., 1588.29 a.u., 2920.88 a.u. and 3937.84 a.u. respectively.
Fluorescence Signals in Animal Experiment. In animal experiment, we characterized GCaMP6s as a fluorescent indicator for reporting neuronal activity in VPM (AP: -3.48, ML: ±2.70, DV: 6.3). The optrode was positioned in place directly above the region of interest by using the stereotaxic arm. At the same time, fluorescence signals were also recorded to find the proper insertion depth that produced maximum fluorescence intensity. After finding the proper insertion depth, the electrical stimulations were applied and the fluorescent Ca2+ signals were recorded simultaneously. The fluorescence rose up during the 20 s electrical stimulation, and the fluorescence increased with the stimulation current, especially in 300 μA current intensity, the fluorescence rose tremendously to approximately two times than baseline fluorescence (**Fig.3**). Besides, the signals during 20 s electrical stimulation were chopped every 333 ms and were averaged together. In this study, we successfully designed an complete DBS photometry system , which also combined with system simulation.

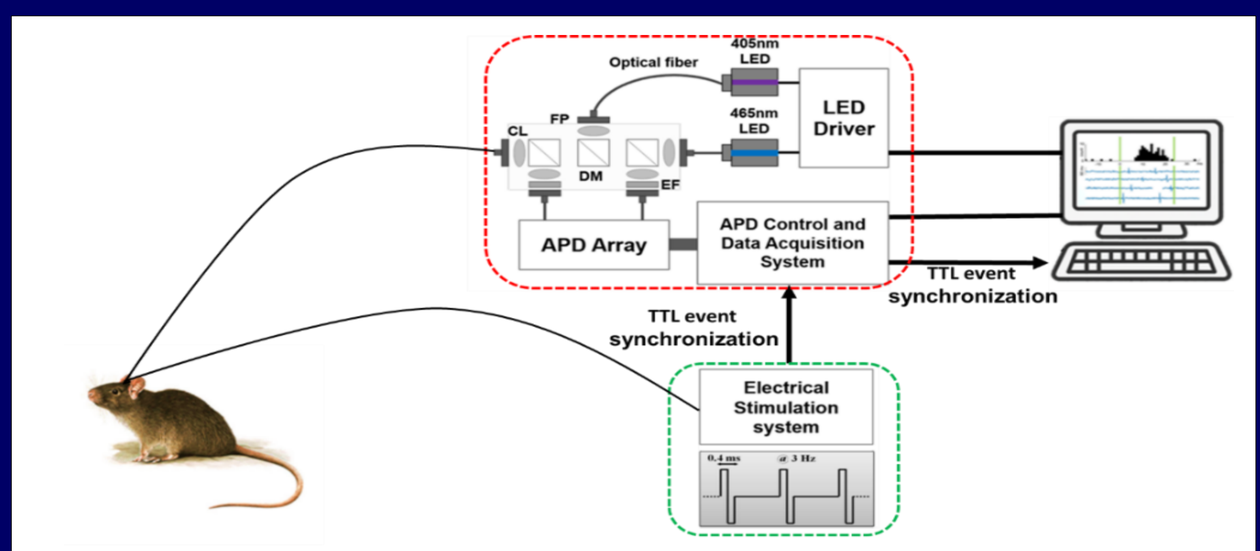

Figure 1 Schematic of the system instrumentation for control and data

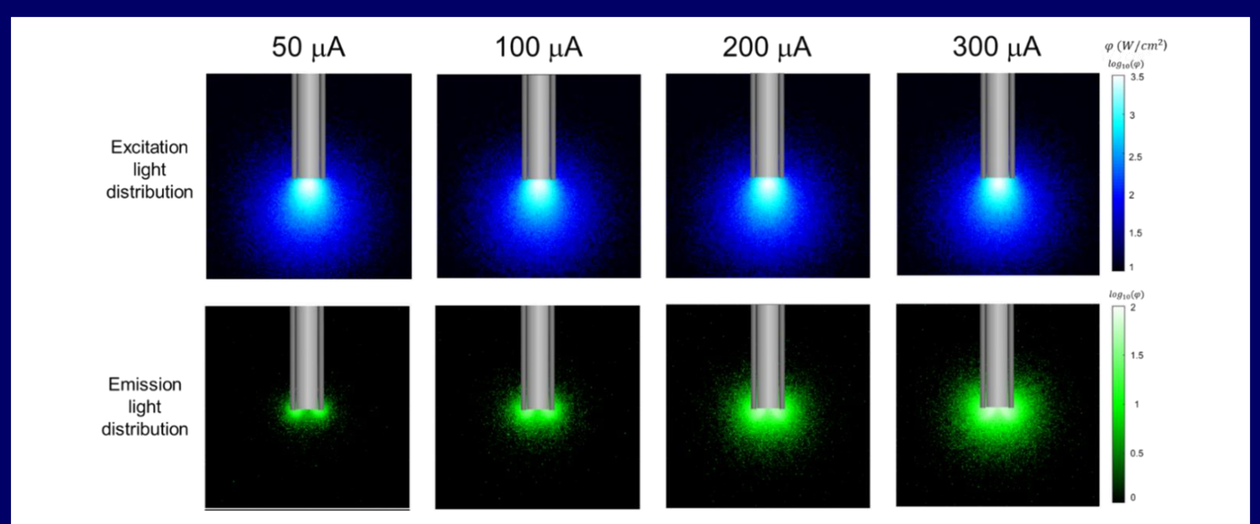

Figure 2 Excitation and emission light distribution in brain tissue under different stimulation current intensities by simulation

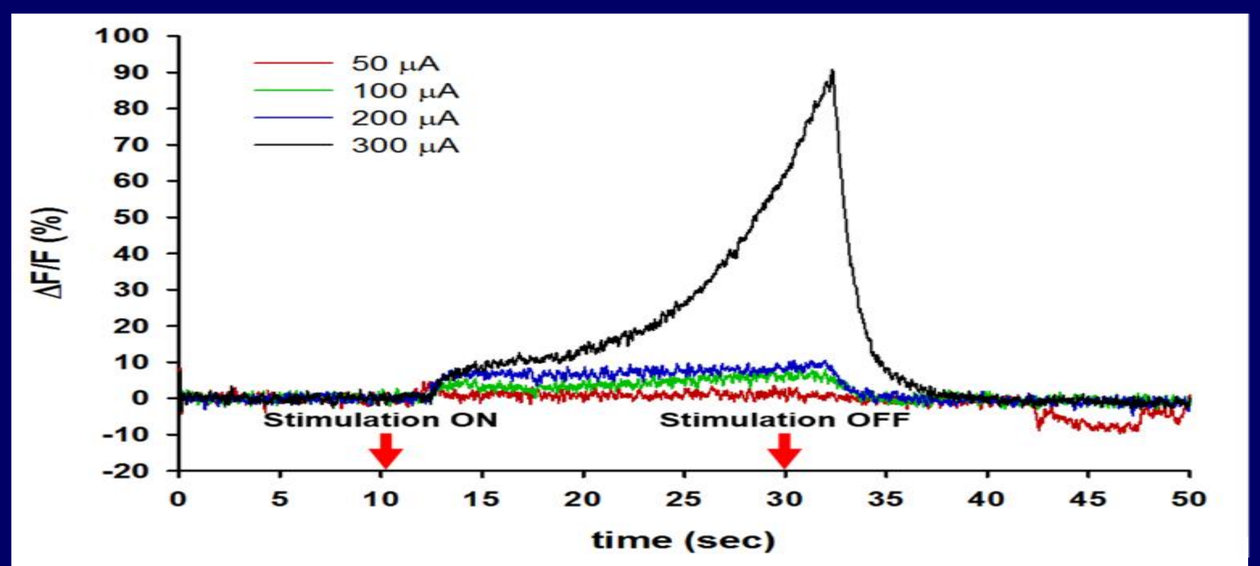

Figure 3 The fluorescence rose up during the electrical stimulation and was increased with stimulation current intensity.

REFERENCES

1) Hubel, D. H., & Wiesel, T. N. (1959). Receptive fields of single neurones in the cat's striate cortex. *The Journal of physiology*, *148*(3), 574-591.
2) Humphrey, D. R., & Schmidt, E. M. (1990). Extracellular single-unit recording methods. In *Neurophysiological techniques* (pp. 1-64). Humana Press.
3) Adelsberger, H., Zainos, A., Alvarez, M., Romo, R., & Konnerth, A. (2014). Local domains of motor cortical activity revealed by fiber-optic calcium recordings in behaving nonhuman primates. *Proceedings of the National Academy of Sciences, 111*(1), 463-468.
4) Ashley, C., & Ridgway, E. (1968). Simultaneous recording of membrane potential, calcium transient and tension in single muscle fibres. *Nature, 219*(5159), 1168.