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Cortical Mapping of the Optically Evoked Responses in Channelrhodopsin-2 Mouse Model

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***Abstract*—Little is known about the information transfer properties of large-scale neural circuit in brain system. We applied optical deep brain stimulation to define the properties of information flow within a living brain assisted by channel rhodopsin-2 (ChR2) transgenic mice, of which neurons express the light-activated ion channel. We first characterized the responses of neuronal ensemble to the impinged light with respect to stimulation parameters by co-registering local field potentials with optical stimulation. Secondly, we applied recently developed polyimide based microarray for mouse electroencephalogram (EEG) to obtain the cortical responses with respect to deep brain stimulation. Particularly, the spatiotemporal cortical mapping with respect to deep brain stimulation of primary somatosensory cortex and hippocampus CA1 were presented in this article.**

I. Introduction

A mouse model has several benefits in studying large-scale functional circuit in brain both at synaptic or molecular levels. The rich resource of genes enables us to modify molecule or neuron to correlate with functional behavior. As a way to study information processing in large-scale brain networks, the methods such as the evoked or event-related brain potential or electrical deep brain stimulation are also applicable to mouse model [1]. Such a direct electrical stimulation method, however, has several demerits including tissue damage, non-selectivity on activation or inhibition, and non-selectivity on neural type. Furthermore, the electromagnetic interference from the stimulator can contaminate the electrophysiological signals.

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Recently introduced technique, called optogenetic method excites or inhibits neurons using light sensitive proteins such as Channelrhodopsin-2 (ChR2) [2] or Halorhodopsin (NpHR) [3]. Since this technique use monochromatic light, the optogenetic stimulation does not interfere with the electrophysiological signals. In addition, the light driven chloride anion pump, NpHR can inhibit the neuron from firing. Furthermore, a usage of cell type specific promoters induce the light sensitive channels pervade into the aimed cells, hence cell specific stimulation is possible with optogenetic method.

In this paper, we use the transgenic mice, Thy1-ChR2-EYFP, of which all neurons express ChR2 proteins and expression area can be confirmed by YFP fluorescence imaging [4]. We are particularly interested in characterizing the dependence of neuronal response on the stimulating parameters such as pulse duration and light power. The secondly activated responses in the neuronal population connected to the firstly stimulated region are mapped especially on cortex using EEG. Being concerned in the complex sensory, motor and cognitive functions, the large-scale networks are closely linked with an amount of separate cortical areas in the cerebral cortex. In our work, we locally stimulated the brain regions such as cerebral cortex and hippocampus with light source delivered by optic fiber connected to laser and then the cortical responses to the optical stimulation were measured with high density EEG using polyimide-based microelectrode (PBM) on mouse skull [5-7]. For detailed information on surgery procedure, see [8].

Many studies have already reported the physical conditions of light stimulation for light-induced neural firing in vitro [9] and in vivo [10], but most of the conditions were designed to satisfy well-operating action potential or spike not neuronal population that can be applicable to alive animals in real time. It is required to quantify the activated responses in terms of stimulation parameters to determine the functional connectivity based on known stimulation condition. To quantify the strength and latency of the local response due to the optical stimulation, we co-localized the low impedance electrode and optical fiber. The electrode acquires the average behavior of nearby neuronal population, which is local field potential (LFP). The convergence technology of optogenetic control and microelectrode electrophysiology successfully delivered us the dynamic and functional brain mapping in cortex evoked by an artifact-free light stimulus which can be greatly beneficial to the current understanding of large-scale brain network.

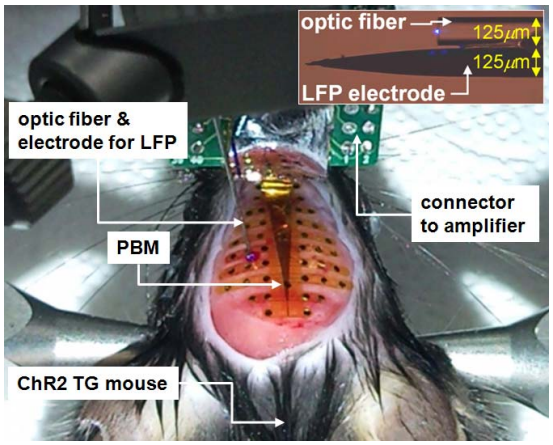

Fig. 1. Experimental set-up. The PBM with 38 channels positioned on the skull. The optic fiber is connected to the semiconductor laser (wavelength = 440 nm) and coupled to LFP electrode. A hole on the skull was made for the penetration of optic fiber and LFP electrode into the brain. The inset figure is a microscopic picture of tips of co-localized optic fiber and LFP electrode.

II. Materials and Methods

A. In vivo light stimulation and electrophysiology

In vivo recordings were performed on ChR2 transgenic mice (30-35 g in weight; 12-15 weeks; male) anesthetized with injected ketamine/xylazine cocktail (120/6 mg/kg, i.p.). The midline of scalp was incised to expose the skull. After wiping the skull, the PBM was attached on the skull. Detailed procedures for fabrication and surgery of PBM are described in [5]. A semiconductor laser (FCLM-440, Oplink Communications Inc., CA., USA) was gated using a pulse generator (575 digital delay, Berkeley Nucleonics Corp., CA, USA) and guided to the brain using an optic fiber with clad/core diameters of 125/3.4 μm, respectively (P1-405A-FC-5, Thorlabs, NJ, USA). The power intensity was modulated by input modulation knob in the laser module and calibrated by the optical power meter (PM100D, Thorlabs, NJ, USA), of which maximal power is 15 mW/mm^2 at the end of optical fiber. The light stimulation was given at a frequency of 5 Hz for 20 sec and each pulse has a variable pulse duration ranging from 1 to 40 ms.

The LFP electrode (UNA35EBR, FHC, ME, USA) was carefully adhered to the fiber using epoxy (404, Loctite®) in a way that the tips are aligned as shown in the inset figure of Fig. 1. A hole was made on the skull for access of the optic fiber and LFP electrode. Figure 1 is a dorsal view of the mouse under stimulation and recording. The LFP and EEG signals were acquired by a SynAmps amplifier (Neuroscan Inc. TX, USA). One electrode on the occipital lobe was used as a ground electrode and one electrode on the olfactory bulb was used as a reference electrode. Impedance between the electrodes and the animal's skull were kept below 300 kΩ. The light triggering signals from pulse generator were also sent to the amplifier to synch the stimulation and electrophysiological data. Animal protocols were approved by the Korea Institute of Science and Technology Animal Care Committee.

B. Data analysis

All data analysis was performed using custom software written in MatLab (Mathworks, MA, USA). As a preprocess, each EEG and LFP channel was divided by normalization factor, which was defined by the average power of range, 130 Hz to 170 Hz from resting period. All the data were segmented into epochs of 250 ms in time windows between 50 ms before and 200 ms after the light onset.

To facilitate the identification of evoked signals that selectively responds to the light stimulus and are not under influence of background brain activity, we excluded the outlier signals in a set of optically evoked signals. The outlier signals were detected by finding a signal whose correlation to the averaged signal is below 0.95. A nonlinear regression analysis based on least square method was performed on the ensemble response of LFP data to find the best fitting function of LFP in terms of optical stimulation parameters.

The ensemble response of 38 EEG channels was mapped over mouse cortex. The brain surface was rendered showing the bregma and lambda points to allow accurate placement of the virtual markers on the 3D-reconstructured magnetic resonance imaging data downloaded from http://www.bnl.gov/ctn/mouse. The potential values were interpolated by cubic spline interpolation method.

III. Results and Discussion

A. Relationship between stimulation parameters and neuronal population responses

To characterize the neuronal population response to the optogenetic stimulation, nonlinear regression was performed on a series of ensemble epoch in LFP with different stimulation parameters. The best fitted function of neuronal response follows Rayleigh distribution as

$$f(t;E,\tau)=\frac{Et}{\tau^2}\exp\left(-\frac{t^2}{2\tau^2}\right), \qquad \text{(Eq. 1)}$$

where E and τ are defined as peak amplitude and peak delay to light stimulation (latency), respectively. This transfer function predicts the behavior of neuronal response for given stimulation parameters. To determine the dependency of E and τ on stimulation parameters of pulse duration and light intensity, we stimulated with variable stimulation conditions: from 0.5 to 15 mW/mm^2 of light stimulation intensity and from 1 to 40 ms stimulation pulse duration. Figure 2 shows the resultant dependency of E and τ on stimulation parameters of pulse duration and light intensity. The peak amplitude, E in LFP response with respect to optogenetic stimulation increase monotonically with pulse duration and light intensity until it is saturated to its maximal response. For example, at a light

intensity of 10 mW/mm^2, E increases as stimulation duration increases until the pulse duration exceeds 20 ms. Whereas the latency τ in LFP response

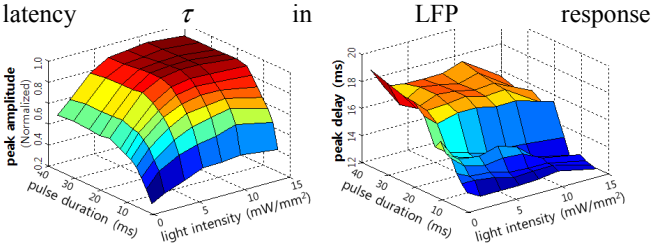

Fig. 2. Functions of peak amplitudes, E and latency, □ of neuronal response in terms of light stimulation parameters, e.g., light intensity and pulse duration.

monotonically increases with respect to pulse duration at given light intensity until τ to be saturated at 17 ms approximately. On the other hand, τdependency on light intensity was marginal except weak light intensity smaller than 1 mW/mm^2. At the extremely low light intensity, for example, at 0.5 mW/mm^2, τ has an unstable feature because of large variance in light-induced activity, therefore a robust light induced neuronal activation is not expected at this light intensity *in vivo* condition. Based on these results, it is suggested that the stimulation condition of pulse duration between 13 and 40 ms and light intensity stronger than 5 mW/mm^2 are optimal to have the same expected values of peak amplitude and latency in the neuronal response *in vivo*.

A. Dynamic cortical mapping of functional connectivity

To characterize the neuronal connectivity to the cortical regions, we applied optical deep brain stimulation and observed the spatiotemporal dynamics patterns in mouse EEG. In particular, we applied the optical stimulation on the primary somatosensory cortex (S1) and hippocampus cornu ammonis (CA1) regions. The stimulation light intensity and pulse duration were 5 mW/mm^2 and 20 ms, respectively.

The cortical EEG topographies evoked by light stimulation on three brain regions in depth give us completely different functional connectivity information over time. Figure 3 shows the cortical responses with respect to the optical stimulation on S1 cortex. The focal responses at the stimulated region were observed and then re-activation at the same region was monitored accompanied by contralateral responses in the parietal cortex. Additionally, the dynamic brain mapping could provide the information of response latency, which is approximately 24 ms in this case. The location of maximal response coincide with the stimulation position and the response amplitude decreases along the distance from stimulation spot in a power law, which is consistent with the photon density of light is decaying in a power law [11]. However, the range of response is larger than the decay constant of photon density, which is less than 1 mm, which is speculated due to the volume conduction.

Figure 4 shows the cortical response to left CA3 hippocampal stimulation. The ipsilateral primary somatosensory cortex of limb region was activated first with latency of about 24 ms and then contralateral primary somatosensory cortex of limb region was activated about 30 ms after the peak response at the ipsilateral region. It is interesting to note that the synaptic response was more anterior than the direct response to the optic stimulation. No direct activation due to volume conduction from the activated region is observed. It means that the hippocampal stimulating region is sufficiently far from the directly recordable range of EEG, hence the EEG signals are supposed to be a result from functionally connected synaptic projection.

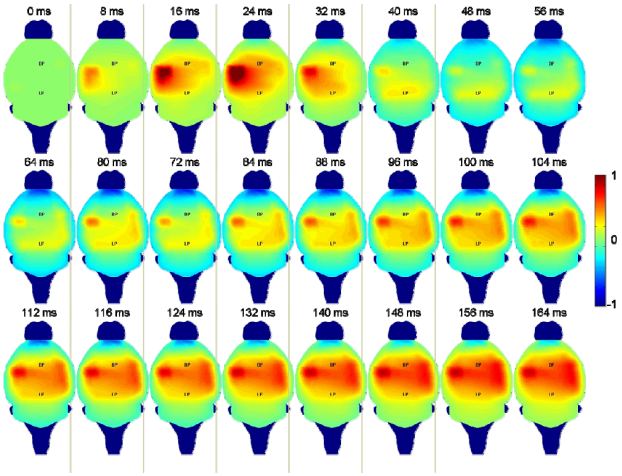

Fig. 3. Cine mode of cortical responses to optical stimulation on left barrel cortex in primary somatosensory cortex. . The pulse duration and light intensity were 20 ms and 5 mW, respectively and the number in the figure denotes the time after the light onset. The values were normalized by the maximal response. Averaged responses with respect to 20 equivalent optical stimuli were plotted. BP and LP in the map stand for the bregma and lambda points, respectively.

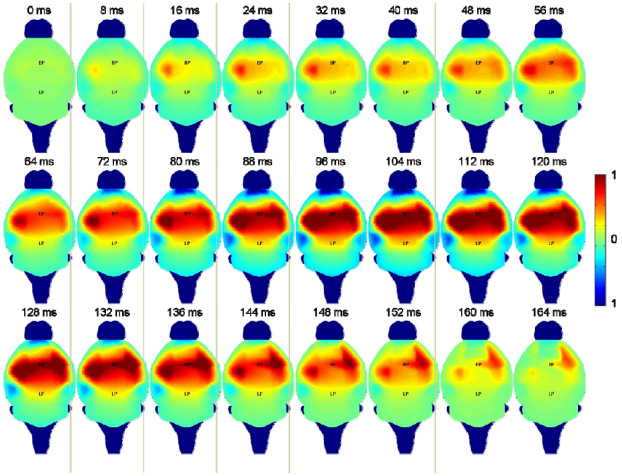

Fig. 4. Cine mode of cortical responses to optical stimulation on left CA1 hippocampus. Descriptions are the same as in caption of Fig. 3.

IV. Conclusion

We demonstrated tracking information flow in a living brain by combining optical stimulation in channelrhodopsin-2 transgenic mice and functional mouse brain mapping using polyimide-based microarray. The co-localized LFP responses shows that the field responses parameters are dependent on light stimulation parameters such as pulse duration and light intensity, and based on the measurement data we built a fitting model predicting the neural response for given stimulation parameters. The temporal mapping of EEG delivered the cortical responses with response to optical stimulation, particularly on cortex and hippocampus. The direct response to optical stimulation was observed in case of cortical stimulation followed by synaptic responses in contralateral region. Not only the latency in the signal transfer but also the strength can be evaluated. The cortical responses with respect to the CA1 hippocampal stimulation show the reciprocal connection between hippocampus and cortex very well. The first cortical response in the ipsilateral limbic region was transferred to the contralateral limbic region presumably via reciprocal connection between hippocampus and cortex.

In this study, the system in this paper can be utilized to characterize any functional circuit related to cognitive behavior in awake animals by perturbing the related brain region using the optogenetic tools and observing the electrophysiological response in cortex directly. This method in this paper is invasive and thus not suited for large scale studies, e.g., on humans but it suggests a new method to define the connectivity from brain region to cortex in high temporal resolution, which can deliver complimentary information to high spatial brain imaging techniques such as non-invasive near infrared spectroscopy, diffusion tensor imaging or functional magnetic imaging

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