HIPPOCAMPAL ACTIVITY DURING SEROTONERGIC NEURONAL ACTIVITY MANIPULATION USING OPTOGENETICS

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***Abstract*—In order to improve brain dysfunction such as mental disease, it is necessary to clarify the mechanism of activity in the brain. To clarify the relationship between the brain region and the neurotransmitter is important for elucidating the brain. Especially investigating the relationship between serotonin and hippocampus leads to elucidate of the mechanism of depression and sleep. The technique called optogenetics enabled only specific cells to be manipulated by light stimulation in recent years. This technique is a useful technique for clarifying the function of the brain. Because instead of stimulating multiple cells such as electrical stimulation, optogenetics can stimulate only specific cells, so that its function can be clarified. This study focused on neurotransmitter serotonin and the brain region called the hippocampus. For these purpose we can clarify the relationship between serotonin and hippocampus by manipulating serotonin neural activity using optogenetics while invasively measuring the ventral hippocampus of the mouse. In addition, identification of pyramidal cell layer is necessary to clarify hippocampal activity. Currently, visual estimation is performed based on the histology that dissected the brain, it still has a problem. Now this technique can't estimate ambiguous of the brain part and measure in an online manner. For this problem, we focus on the clarity of the brain part estimation when we used optogenetics. Therefore, we could estimate the pyramidal cell layer of the hippocampus by analyzing nerve activity and thought that the effect of serotonin activation or suppression on the hippocampus could be clarified. As a result, we showed that pyramidal cell layer can be identified by using principal component analysis, and could clarify the variation of nerve activity in the pyramidal cell layer of hippocampus during serotonin operation.**

I. INTRODUCTION

Activity in the brain is formed by neurotransmitter transmitting information from each brain region. It is thought that various dysfunction such as dementia, insomnia and depression caused by the deterioration of the functions of these activities. For this reason, study to elucidate the function of each brain site and the role of neurotransmitter is important to ameliorate these disorders. In this study, we focused on the hippocampus, one of the brain regions, and investigated the hippocampal neural activity by manipulating serotonin. The hippocampus exists the temporal lobe inside of the cerebral cortex and is part of the limbic system. The hippocampal activity plays an essential role in learning, memory, emotion, and regulation of stress response, and it is regarded as an indispensable part for episode memory and emotional memory formation concerning individual experience.

In recent studies, hippocampus is related to psychiatric disorders. Depression and post-traumatic stress disorder (PTSD) is related to hippocampal atrophy [1]. The research using mouse, hippocampus is named ventral and dorsal by means of their data acquisition position. In experiment, it was revealed that when the ventral hippocampus was excised, spatial memory was maintained, but the anxiety - related behavior was lost as the freezing response in the fear conditioning experiment decreased. And it has been clarified that in the case of resecting the dorsal hippocampus it causes obstacles to spatial learning. It is believed that the ventral and dorsal hippocampus is strongly related to emotion and spatial cognition, respectively [2, 3]. In addition, it is clear that raising the concentration of monoamine in the brain change for the better depression, and correlates with decrease in serotonin due to the correlation of decrease in serotonin neuronal activity [4, 5]. In other words, it produces an anxiolytic effect by activating serotonin nerve. By the way, serotonin is related to the effect of suppressing REM sleep and wakefulness / sleep cycle [6], and have a deep connection with the sleep mechanism [7]. Serotonergic neurons are clearly related to functions that are important for human living. It is important to quantitatively evaluate and elucidate the relationship between hippocampus and serotonin from the viewpoint of depression and sleep, and expected to improve psychiatric disorders and improve the sleep quality by manipulating serotonin.

In general, hippocampal neuronal activities is analyzed using multichannel electrodes against mouse brain, but it is difficult to unify the measurement site because the shape of the brain is somewhat different in individuals. It is indispensable to estimate the position of the pyramidal cell layer of the hippocampus. Visual estimation is performed based on the histology by dissecting the brain after the experiment. This estimation is an ambiguous indicator and only can be used to estimate one measurement per mouse. We considered that the hippocampal pyramidal cell layer can be estimated by using the hippocampal neuronal activity and we can reveal the pyramidal cell activity fluctuation. By doing this, it is possible to estimate each time even if a single mouse performs multiple measurements.

In this study, we propose an estimation method of pyramidal cell layer and clarify the effect of serotonin on hippocampus for clarify cause of mental disease and sleep mechanism.

II. Optogenetics

Optogenetics is one of the experimental technique in neuroscience that combines tissue and cell type-specific expression of light sensitive proteins called opsins and advanced optical methods to reach, record, and control the activity of a specific cell population. By expressing light sensitive proteins in specific cells, it becomes possible to manipulate only by light stimulation [8]. In the study on neuroscience using this technique, it is main stream to use animals such as mouse. Conventional experimental techniques have used electrical stimulation to force cells in the brain region to be activated, knockout mouse invalidating (knocking out) specific cell activities, and transgenic mouse to which artificially introduced foreign genes have been introduced. Brain functions have been elucidated by comparing these methods with normal mouse. However, these techniques are difficult to stimulate only specific cells, and there is a disadvantage that the knocked out portion can't be returned to the normal state. On the other hand, the major feature of the current optogenetic technology is that it can be used for live animals and only specific cells can be manipulated (Fig. 1). Therefore, since the problem that it is difficult to associate behavior with brain and nervous functions and impossible to manipulate specific cells like electrical stimulation has been solved, it is possible to obtain association with behavior and to clarify the mechanism of the brain and nerve function. Elucidation of the mechanism which could not be clarified by conventional methods such as study on memory operation by light and study on remuneration behavior by serotonin suppression has dramatically advanced by this technology [9, 10].

A light sensitive protein which is indispensable for exploiting the optogenetic technology can be activated or suppressed in order to cause hyperpolarization and depolarization of expressed cells upon light stimulation. Currently, it is roughly divided into three kinds, channelrhodopsin, halorhodopsin and archaerhodopsin. These have different wavelengths of light to be activated and functions at the time of activation (Fig. 2). A light sensitive protein has the feature that it does not affect the activity even

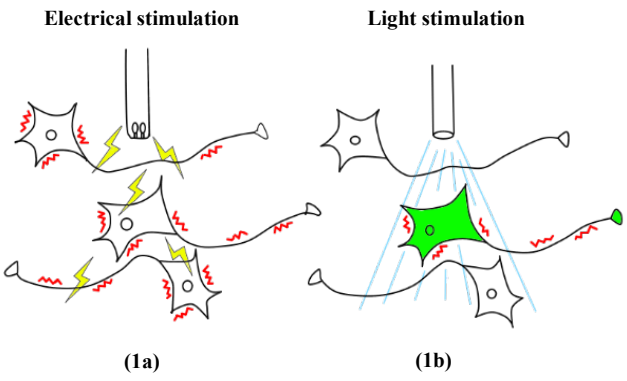

Fig. 1. Cell stimulation method. (1a) Electrical stimulation stimulates peripheral cells. By using this electrical method, only specific cells are not activated. (1b) Light stimulation by using optogenetics. Owing to this technique, only specific cells are activated.

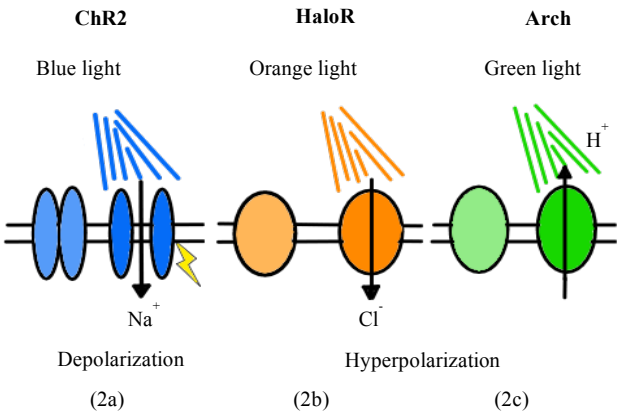

Fig. 2. Light sensitive proteins. (2a) Channelrhodopsin (ChR2) is activated by receiving blue light. The membrane potential is depolarized by blue light irradiation when it is expressed on neurons, and the cellular activity is excited. (2b) Halorhodopsin (HaloR) is activated by receiving orange light. It is expressed in nerve cells when the membrane potential is hyperpolarized by orange light irradiation, and cellular activity is suppressed. (2c) Archaerhodopsin (Arch) is activated by green light. Similarly to halorhodopsin, cell activity is suppressed because the membrane potential is hyperpolarized by irradiation with green light.

if it is introduced into a normal cell because it does not function while light is not irradiated.

This study aims to investigate the change of nerve activity of hippocampus by manipulating serotonin nerve using optogenetics. This study is possible to know the influence on the hippocampus based on the activity of serotonergic nerve by clarifying the activity of the hippocampus at the time of activation and suppression of serotonergic neurons, so we can elucidate mechanism of the hippocampal activity.

III. Experiments

A. Experimental Preparation

In this experiment, two types of mouse capable of manipulating the dorsal raphe nucleus of the serotonin nervous system are used. One was to express channelrhodopsin which brings about an activation effect by light and the other to express archaerhodopsin which has an inhibitory effect. In order to perform the measurement, a mouse was used which had a head plate, an optical fiber, a reference, a ground, and a cranial removal operation to invade the measuring instrument to the brain. Optical fibers were installed near the dorsal raphe nucleus from the occipital region so that light irradiation could be performed. The operation for preparing these experiments is performed more than one week before the measurement day, and measurement is carried out after establishing a recovery period.

B. Experimental Method

The purpose of this study is to elucidate the hippocampal neuronal activity during serotonin manipulation using optogenetics. The object to be measured in this experiment was the ventral hippocampus because it is said to be related to emotional memory and considered to have a connection with serotonin (Fig. 3). Since using multiple channels can specify

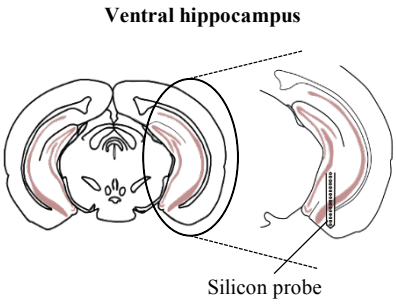

Fig. 3. The ventral hippocampus which is the measurement part of this experiment. As shown in the figure invade the silicon probe.

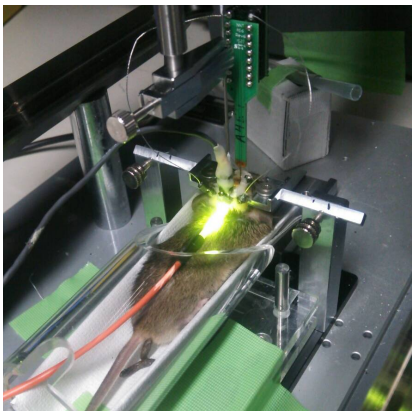

Fig. 4. Experimental landscape. The appearance that the head-fixed mouse is measured while light irradiation.

the pyramidal, we measured synaptic potential using a 16-channel silicon probe. Synaptic potential measurement was started after the silicon probe was invaded to the hippocampus of the abdomen on a head fixed to the head (Fig. 4). Measurement time was about 2280 seconds, and activation and suppression of serotonin for 30 seconds by light irradiation were performed 10 times in total during measurement. An interval of about 180 seconds was made from the operation period to the next operation period. In the case of channelrhodopsin, the activated state is maintained after blue light irradiation and it is released with yellow light. Blue light irradiation for 0.5 seconds and activation with yellow light for 0.5 seconds after 30 seconds are activated for 30 seconds in this experiment. Archaerhodopsin can be suppressed while applying yellow light. So irradiation for 30 seconds is continued for suppression for 30 seconds in this experiment (Fig. 5).

IV. Analysis

In this study, the pyramidal cell layer was estimated from the principal component analysis of the 16 channel data obtained at the beginning. Next, the time series data of the neural activity obtained from the measurement was converted to the frequency domain, and the activities of the pyramidal cell layer at the time of serotonin operation and without operation were compared.

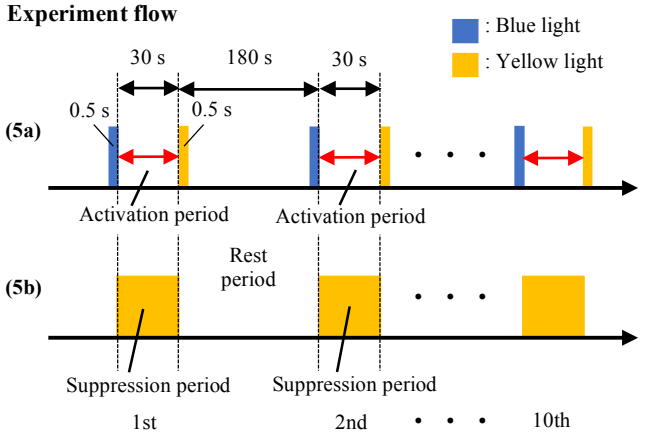

Fig. 5. Serotonin operation under measurement. (5a), case of activating serotonin nerve by channelrhodopsin. (5b), case of inhibiting serotonin neurons by archaerhodopsin.

A. Estimation of Pyramidal Cell Layer

It is said that the phase of the signal is reversed in the electrode placed in the pyramidal cell layer [11]. Therefore, pyramidal cells could be estimated by performing principal component analysis and correlation analysis. Principal component analysis is a multivariate analysis technique that synthesizes variables called principal components that best represent overall variability with a small number of uncorrelated from many correlated variables. We define the channel that low coefficient value of the first principal component and the large coefficient value of the second principal component is placed in the pyramidal cell layer when the data obtained from 16 channels is divided into the first principal component and the second principal component. In addition, since the phase of the pyramidal cell layer is reversed, the channel having the low correlation value is defined as a pyramidal cell layer by calculating the correlation coefficient between the surroundings of each channel. A correlation coefficient is calculated by the following equation.

$$\rho(A,B) = \frac{1}{N-1}\sum_{i=1}^{N}\left(\frac{\overline{A_i - \mu_A}}{\sigma_A}\right)\left(\frac{B_i - \mu_B}{\sigma_B}\right) \qquad (1)$$

where ρ, N, μ_A, σ_A, μ_B, and σ_B indicate the correlation coefficient, observation value average of A, standard deviation of A, average of B, and standard deviation of B, respectively.

B. Neural Activity Analysis

30 seconds during which serotonin nerve is manipulated by light irradiation is in an activated state or suppressed state, and 180 seconds before irradiation is normal state. Frequency analysis in these conditions was performed. The acquired data has a spike potential (around 1 kHz) of cells called unit activity and a local field potential (1-250 Hz). In addition, hippocampus of rodent has θ wave (4-10 Hz) higher than normal θ wave (4-8 Hz), γ wave (25-140 Hz), and HFO (140-200 Hz, High Frequency Oscillation). Therefore, extra frequency was removed by using a bandpass filter of 1-200

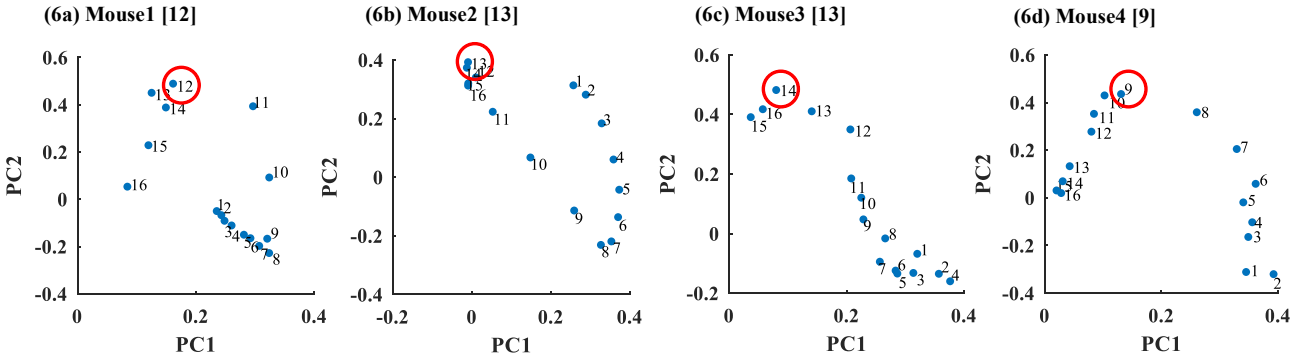

Fig. 6. The results of principal component analysis, x label is principal component coefficient of PC1 and y label is PC2. Plot number is channel number. The red circle is a channel considered to be a pyramidal cell layer. Numbers in brackets of title is an estimate of pyramidal cell layer by histology.

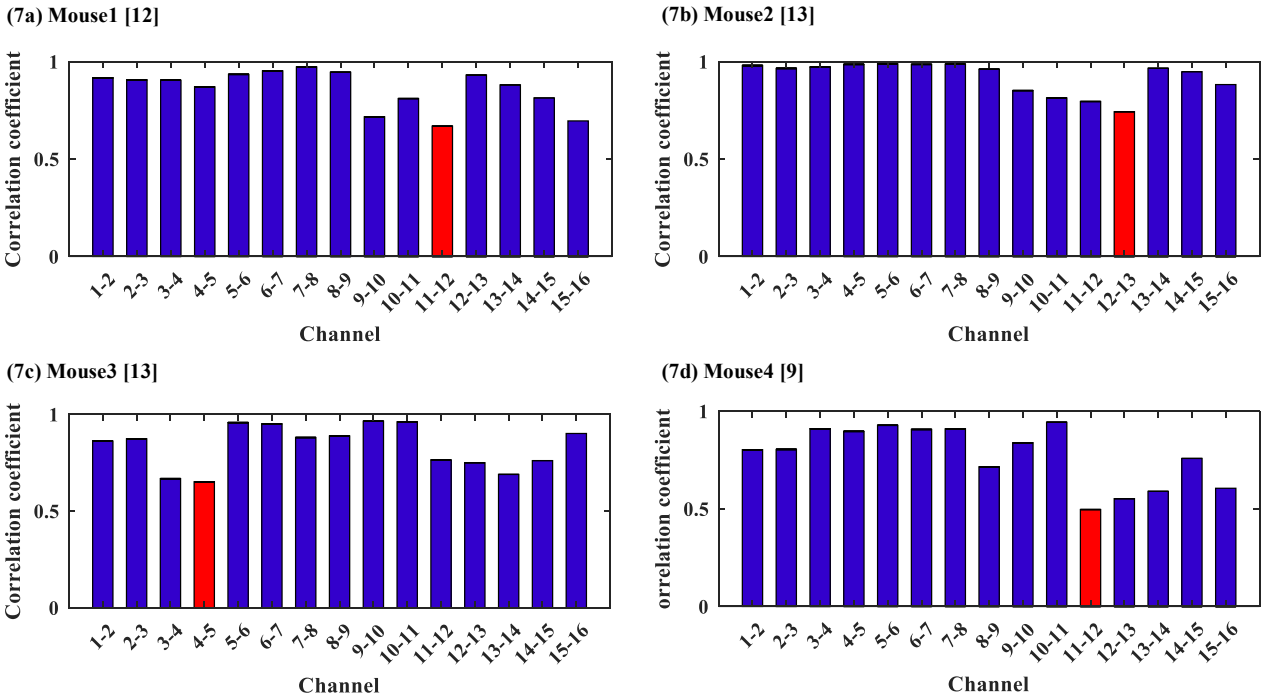

Fig. 7. The results of correlation analysis, x label is compared channel and y label is correlation coefficient. The red bar graph is a channel considered to be a pyramidal cell layer. Numbers in brackets of title is an estimate of pyramidal cell layer by histology.

Hz in the preprocessing because considering the local field potential up to HFO. Finally, normalization was performed to take into account individual differences of mouse. After the preprocessing, amplitude spectrum was calculated by transforming to frequency domain using fast fourier transform (FFT). Firstly, we calculated the coefficient of variation of the amplitude spectrum from the normal state to the activated state or suppressed state for each mouse and investigated the variety by serotonin operation. Then, we can clarify the influence of serotonin on the pyramidal cell layer by covering all the mouse with the channel of the pyramidal cell layer as a reference.

V. Results and Discussion

The results of principal component analysis and correlation analysis are shown in Fig.6, 7. Numbers in brackets of title is an estimate of pyramidal cell layer by histology and we made this channel of numbers correct. Thus, Fig. 6 shows the possibility that the channel in red circle is a pyramidal cell layer. In contrast to this, in the case of Fig. 7 shows the possibility that a red bar graph is a pyramidal cell layer because it is the minimum correlation coefficient, but different results from histology is obtain as (7c) and (7d). Because the ventral hippocampus has a complicated layer structure, so the possibility of occurrence of a channel with lower correlation due to passage through layers other than the pyramidal cell layer, and correlation value of pyramidal cell layer increased since the presence of multiple channels near the pyramidal cell layer. From these results, estimation of the pyramidal cell layer by the principal component analysis is effective, which is considered to be a new quantitative estimation method.

Moreover, we show the results of frequency analysis of activity of hippocampus during serotonin operation. The

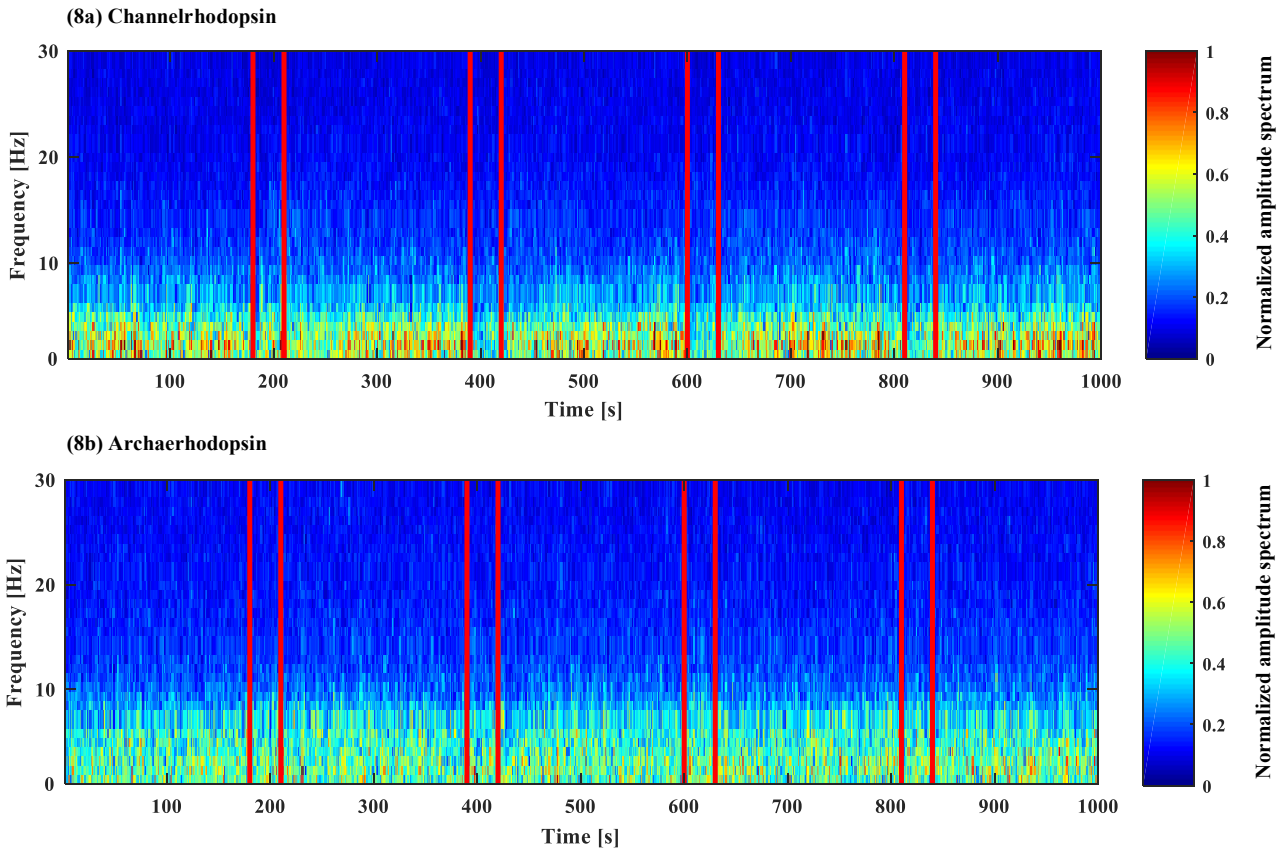

Fig. 8. Amplitude spectrum at each elapsed time. This figure shows up to four irradiations. Serotonin operation is performed during the narrow interval from the red line to the next red line. (8a), the amplitude spectrum of the low frequency part is decreased by serotonin operation.

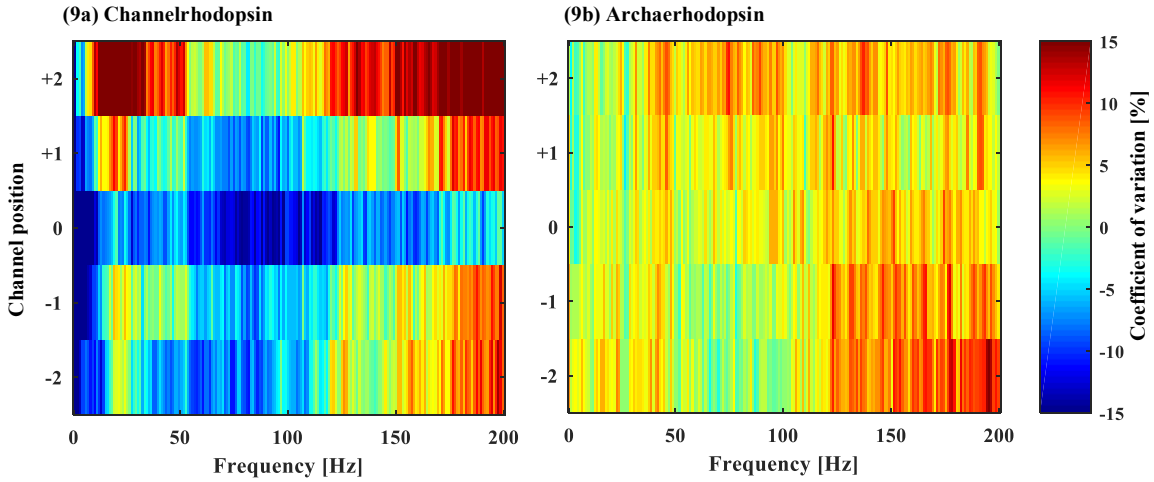

Fig. 9. The results of calculating the variation rate based on pyramidal cell layer. Zero is the pyramidal cell layer, plus is deep brain, and minus is brain surface. (9a), the amplitude spectrum of pyramidal cell layer decreases due to serotonin activation. (9b), the amplitude spectrum of pyramidal cell layer increases due to serotonin suppression.

calculation result of the rate of change by serotonin operation for each mouse showed a tendency that the amplitude spectrum value of HFO increased and the fast γ band at 60-120 Hz decreased in both cases serotonin was activated and suppressed. It is thought that it is not a fluctuation due to the activity of serotonin nerve but an activity fluctuation due to light stimulation because the same result was obtained not only at activation but also at suppression. Furthermore, the analysis result of the channel estimated to be a pyramidal cell layer by principal component analysis is as follows. Fig. 8 shows the result of displaying the frequency analysis in chronological order. From this result, a decrease in the amplitude spectrum of the low frequency of the hippocampus when the serotonin was activated was confirmed, but no

significant change was observed when suppressing serotonin. We thought that this result seems to have strong influence of channelrhodopsin on activity of hippocampus than archaerhodopsin, the influence of serotonin on the hippocampus is only when it is secreted and may not have a big influence when the amount of secretion is small, and there was insufficient irradiation time until archarodopsin suppressed serotonergic nerve. Fig. 9 shows the fluctuation rate of neural activity obtained with the channel moved forward and backward by 2ch with reference to the pyramidal cell layer. From these results, it was confirmed that only activation of the pyramidal cell layer was decreased when serotonin nerve was activated, and it was found that activity was increased to some extent when suppressed.

VI. CONCLUSIONS

The purpose of this study was to estimate hippocampal pyramidal cell layer by analyzing nerve activity and to clarify hippocampal activity in serotonin operation. As a result, estimation of pyramidal cell layer by principal component analysis was considered to be effective. Our contribution is as follows:

- Estimation of pyramidal cell layer by principal component analysis was considered to be effective.
- In serotonin manipulation using optogenetics, activity change of the hippocampus when activation of serotonin nerve by channelrhodopsin was remarkable, and conversely, there was not much change observed by suppression of archaerhodopsin.
- Compared the activity during serotonin manipulation with the normal state, so we could confirm a negative correlation with the activity of pyramidal cell layer in the hippocampus and serotonin.

In the future, it is necessary to prove that it is more reliable by increasing the number of mouse to be verified. In addition, we intend to study the relation between the hippocampus and serotonin by correlating with the behavior so as to study this relation from the viewpoint of the neuroscience and to investigate the causes of fluctuation due to deviations of measurement points.

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