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The analysis of dynamics and the relationship between spontaneous and evoked activity as cell assemblies in a cultured neuronal network*

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Abstract— **In a brain, it is considered that the synchronous activity of neurons expresses a representation of information. Hebb named the synchronous active group of neurons "Cell Assembly". In this study, we hypothesized that a repeatedly expressed pattern is a cell assembly representing a certain kind of information and attempted to extract such "Cell Assembly" by X-means clustering based on spatiotemporal continuity of spontaneous spikes. Moreover, we divided cell assemblies into classes consist of similar types of cell assemblies, using the indiscernibility-based clustering, "rough clustering". As the result, it showed that cell assemblies did not have a large temporal extent, but a spatial extent. Additionally, we analyzed the neuronal network activity as the stochastic process whose state space is the set of cell assembly, finding out that similar patterns appear consecutively. According to these results, information processing in the neuronal network is suggested to be the hierarchical process. Finally, the clustering method was adopted for spontaneous activity and the evoked responses. It is suggested that spontaneous activities and evoked responses are not completely independent, but share resemble activities.**

I. INTRODUCTION

In a brain, it is considered that a set of synchronous activity of multiple neurons, called "Cell Assembly" by Hebb [1], represents a certain kind of information. Therefore, defining and analyzing the activity pattern of neuronal networks is important for understanding the information processing in a brain. In studies with the multi-electrodes-array in which spike trains were analyzed in fixed time-window-width, the feature vector whose elements were numbers of spikes in each time-window was considered to express a certain activity pattern. Similarly, a method to obtain cell assemblies based on synchronism or correlation between channels also divided spike trains. Otherwise, it is supposed that each active pattern has various durations due to the difference in the number of cells or synaptic delays, thus these methods with fixed time-window-width may not be able to extract appropriate activity patterns. In this study, we attempted to obtain cell assemblies, using X-means clustering based on spatiotemporal continuity. Note that "cell assembly" does not mean a population of cells, but the set of spikes, in this study. Moreover, to classify cell assemblies appearing repeatedly over time into a group (cell assembly class), we also applied another clustering to extracted cell assemblies. Using obtained cell assembly classes, we analyzed the dynamics of neuronal network activities.

We also analyzed the relationship between spontaneous activity and the responses evoked by stimulation. Recent research showed that the evoked responses depended on the interval between the latest ongoing network burst (NB) and stimulation timing [2]. Pasquale et al. also indicated that the leading channels of spontaneous and evoked NB were similar [3]. We attempted to elucidate the similarity between spontaneous and evoked activity patterns as cell assembly classes.

II. MATERIALS AND METHODS

A. A neuronal network in a dissociated culture

This study was conducted by the procedures for animal experiments in accordance with the "Kwansei Gakuin University Animal Experiment Management Regulations" and with the approval of the Animal Experiment Committee. In this study, we used cultured neuronal networks of hippocampal cells of fetal rats on embryonic day 18 (E18) from Wistar Rat (Jcl: Wistar, CLEA Japan).

The hippocampal region of fetal rats excised from the cerebrum was decanted three times with glucose-containing PBS^{-}. Trypsin-EDTA (Thermo Fisher Scientific) was added and the cells were shaken for 15 min in a water bath at 37 $^{\circ}$ C. After trypsin treatment, cells were seeded in the cloning ring placed on each MED probe at a density of 7800 cells/mm^2. An extracellular multipoint measurement system (MED64 system, Alpha MED Scientific) was used to analyze the electrical activity of the neuronal network [4]. The MED64 system consists of a MED connector, a head amplifier, and a MED probe. The electrical activity measured by the microelectrodes on the MED probe flows through the MED connector to the head amplifier and is amplified. The electrical signals are then digitized with a sampling frequency of 20 kHz and a quantum number of 12 bits, and stored on a hard disk. The digital signals are analyzed using Spike Recorder (SPR), a program developed in our laboratory. Activity spikes were detected from the extracellular potential data measured by the MED64 system and the SPR program, using the Spike Counter (SPC) program also developed in our laboratory. Spikes are detected when the amplitude of electric potential exceeds a certain threshold empirically determined from the mean and standard deviation of the electric signals. In this study,

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we recorded the spontaneous activity of the neuronal network for 10 minutes. The evoked responses were recorded for 60 minutes. The two stimulating electrodes detected active spontaneous activity were selected. In the neuronal networks, stimulation at short time intervals is suggested to cause hysteresis, in which the previous inputs influence the following activities [5]. Therefore, we stimulated at an interval of 10 s, which is considered not to occur the hysteresis.

B. Extracting Cell Assemblies

We translated the time stamp data obtained from cultured neuronal networks to three-dimensional feature vectors, and extracted cell assemblies by X-means clustering applied on the three-dimensional space. Because applying the clustering for entire data was unable to extract adequate patterns in shortly expected duration, we previously divided data into some batches. The optimal dividing time dt^* was decided by

$$dt^* = min\{dt_i \,|\, c_i - c_{i-1} \leq 0,\ c_{i+1} - c_i \geq 0\},$$

where c_i is assumed to the number of cell assemblies extracted in dt_i assumed to a certain width ($dt_i \in \{25, 50, ...500\}$) of a fixed time window.

C. Classification Cell Assemblies

1) Center Clustering: We assumed similar patterns of cell assemblies had the similar center of gravity, and calculated center of gravity of each cell assemblies. Projecting the vectors corresponding to the center of gravity to two-dimensional space, X-means clustering was applied to these projected centers of gravity. Extracted clusters were classes including resemble patterns of cell assemblies.

2) Clustering applied to point sequences for the cell assembly: We classify extracted cell assemblies with the novel criterion. Each cell assembly was expressed as a point sequence of the active-channel coordinates of spikes included in the cell assembly. The dissimilarity $d_c(x,y)$ between cell assemblies x, y is defined by

$$d_c(x,y) := \min_{y' \in \mathscr{Y}} \left(\sum_{i=1}^{n} d_u(x_i, y'_i)\right) + \frac{m-n}{m}$$

where d_u is euclidean distance, n and m, is the length of x and y, respectively, $\mathscr{Y}$ is the set of subsequences of y with the length of n. d_c certainly satisfies the "identity of indiscernibles", "symmetry", and "non-negativity", but does not satisfy the triangle inequality, therefore it is semimetric in the sequence space. We use the indiscernibility-based clustering, called "rough clustering" [6]. It is the clustering method that constructs the residue classes for each data by similarity, classifying the data through the commonality among all residue classes. The algorithm of rough clustering is shown below.

1) Let X and D be set $\{x_1, x_2, ...x_n\} (n \in \mathbb{N})$ and the dissimilarity matrix, respectively.
2) Divide X by the equivalence relations $R_i (i = 1, 2, ...n)$.

$$X/R_i := \{P_i, X - P_i\}$$

$$P_i := \{x_j \in X \,|\, D_{i,j} \leq Thd_i\}$$

3) Calculate the indiscernibility matrix γ.

$$\gamma(x_i, x_j) := \frac{\sum_{k=1}^{n} \delta_k^{indis}(x_i, x_j)}{\sum_{k=1}^{n} \delta_k^{indis}(x_i, x_j) + \sum_{k=1}^{n} \delta_k^{dis}(x_i, x_j)}$$

$$\delta_k^{indis}(x_i, x_j) := \begin{cases} 1, & x_i, x_j, x_k \in P_k \\ 0, & otherwise \end{cases}$$

$$\delta_k^{dis}(x_i, x_j) := \begin{cases} 1, & x_i \in P_k, x_j \in X - P_k \, or \\ & x_i \in X - P_k, x_j \in P_k \\ 0, & otherwise \end{cases}$$

4) Divide X by new equivalence relations R'_i.

$$X/R'_i := \{P'_i, X - P'_i\}$$

$$P'_i := \{x_j \in X \,|\, \gamma(x_i, x_j) \geq th\}$$

5) Iterate the refinement process 3 and 4 until the indiscernibility matrix become stable, using the same th

Note that Thd_i and th are the thresholds for dissimilarity and indiscernibility, respectively. Th_i is decided as follows.

1) Let D_i be sorted $\{D_{i,j}\}_{j=1,2,...n}$.
2) Calculating $D'_i = \{D_{i,j} - D_{i,j-1}\}_{j=2,3,...n}$, Let *index* be $min\{argmin D'_i\}$
3) Let Thd_i be the average of $D_{i,index}$ and $D_{i,index+1}$.

th is decided arbitrarily. Large th allows tight clustering, while small th allows loose clustering. In this study, we applied the rough clustering to the set of cell assemblies, using dissimilarity d_c.

3) Extracting Exceedingly Similar Cell Assemblies: It was unable to adequately extract similar patterns using any clustering method, therefore we attempted to classify the data into classes constructed by exceedingly similar cell assemblies. We obtained the same cell assemblies as sequences, applying the rough clustering with high th to the set of patterns that appeared more than a certain number. Thus exceedingly tight clustering was achieved by allowing the cell assemblies with only no or a small number of firing electrode channels to escape detection.

D. Analyzing Neuronal Activity As Stochastic Processes

A cultured neuronal network was expressed as the stochastic process whose state space is the *set of cell assembly classes*, defined by analyzing the state transition probability between two cell assembly classes. We analyzed the probability distribution of $P(X_{n+1} \,|\, X_n)$ where $\mathbf{X}$ is the stochastic process $(X_n), (X_n \in S)$, and S is the set of cell assembly classes. Moreover, the cultured neuronal network was expressed as the stochastic process whose state space is the *set of cell assemblies*, defined by analyzing the state transition distance between two cell assemblies each transition step. We also analyzed the distribution of $d_c(X'_n, X'_{n+1})$ where $\mathbf{X}'$ is the stochastic process (X'_n), $(X'_n \in S')$, and S' is the set of cell assemblies.

E. Similarity between Spontaneous Activity and Evoked Responses

To investigate the similarity between spontaneous activity and evoked responses in the neuronal network, we adapted the method described in II-C.3 to cell assemblies extracted from spontaneous activity and cell assemblies extracted from responses evoked by a stimulation electrode was stimulated.

III. RESULTS AND DISCUSSIONS

A. The Spatial-temporal Characteristics of Cell Assemblies

The optimal-dividing-time-window decided by method II-B was 210.86 ± 108.87 ms (mean $\pm$ SD, N=31). We analyzed the duration, the number of spikes, and the diameter of cell assemblies for each culture to observe the spatial-temporal characteristics (Fig. 1). The diameter is the maximum value of the distance between any channels included in the cell assembly. The mean of the duration, the number of spikes, and the diameter were 24.40 ± 15.93 ms (mean$\pm$ SD, N=31), 7.88 ± 3.11(mean $\pm$ SD, N=31), and 5.92 ± 1.25 (mean $\pm$ SD, N=31), respectively. The percentage of the average duration to optimal dividing time window was 0.068 ± 0.039 (mean $\pm$ SD, N=31). It suggested that cell assemblies didn't have temporal extent, but had spatial extent.

B. Classification of Cell Assemblies

X-means clustering was applied to the 2D-projected spatial distribution of the gravity centers of extracted cell assemblies to obtain cell assembly classes (Fig. 2). Consequently, clear classes were not obtained even though the partly similar cell assembly classes were obtained. That is because similar cell assemblies have similar centers of gravity, however, cell assemblies with a similar center are not necessarily similar to each other. The clustering

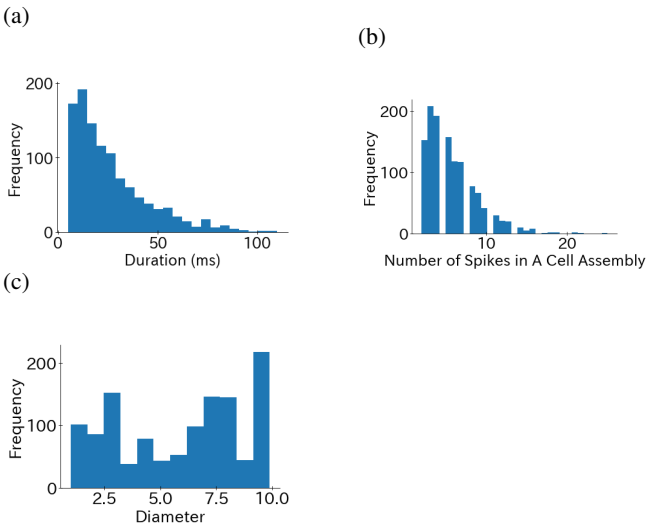

Fig. 1: (a) An example of the distribution of the duration of cell assemblies. (b) An example of the distribution of the number of spikes in cell assemblies.(c) An example of the distribution of the diameter of cell assemblies (E18DIV41).

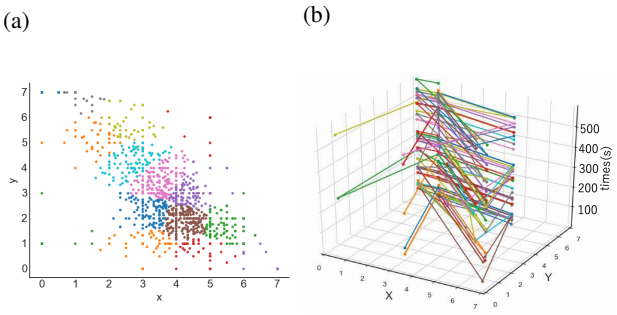

Fig. 2: (a) An example of the distribution of gravity centers of extracted cell assemblies. (b)Example of extracted cell assemblies extracted by applying clustering to 2D-projected gravity centers. Each color shows an individual cell assembly (E18DIV64).

method with gravity centers is suggested as the suitable one for rough classification, in contrast, it is not effective for extracting clear cell assembly classes. The existence of different activity patterns even when the centers are similar indicates that each neuron is involved in multiple types of information processing.

To obtain the more clear cell assembly classes, rough clustering with semimetric d_c was applied to the set of cell assemblies as point sequences. The set of cell assemblies was previously divided by the clustering by centers to reduce the amount of calculation. However, adequate clusters were not obtained even with this method (Fig. 3). Therefore, applying tight rough clustering to the set of cell assemblies appearing more than three times, we attempted to obtain cell assembly classes composed of exceedingly similar cell assemblies. We adjusted th to 0.9 empirically. As a result, certain classes of patterns with few different components (active electrode channels) were extracted in cell assembly classes (Fig. 4a). To evaluate this clustering method, we calculated two values. The two values, *di* and *de* are defined as below.

$$d_i = \frac{1}{n(n-1)} \sum_{x_1 \in X_i} \sum_{x_2 \in X_i} d_c(x_1, x_2)$$

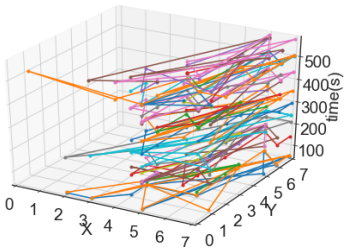

Fig. 3: An example of cell assemblies extracted by the rough clustering. Each color shows an individual cell assembly (E18DIV64).

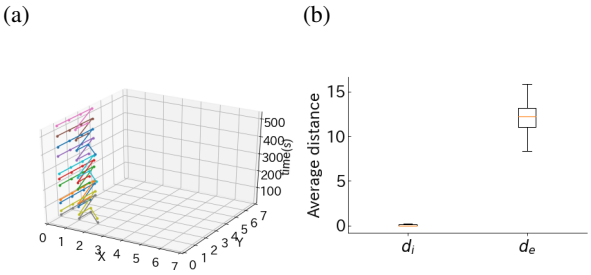

Fig. 4: (a) An example of extracted cell assemblies by tight-rough-clustering. (b) Averaged values of d_i (left) and d_e (right) (E18DIV64).

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$$d_e = \frac{1}{nm} \sum_{x_1 \in X_i} \sum_{x_2 \in X \setminus X_i} d_c(x_1, x_2)$$

, where X is the set of cell assemblies, n and m are the number of cell assemblies in X_i and $X \setminus X_i$, respectively. As the result, d_i and d_i were 0.13 ± 0.15 (mean $\pm$ SD, N=31) and 8.07 ± 3.22 (mean $\pm$ SD, N=31), respectively (Fig. 4b). The clear difference between these values suggested that tight-rough-clustering was effective to extract exceedingly similar cell assemblies.

C. Dynamics of Cell Assemblies

We analyzed the cultured neuronal activities as the stochastic process whose state space is the cell assembly classes. The average transition probability was 0.257 ± 0.112(mean $\pm$ SD, N=31). Moreover, the average transition distance was 9.82 ± 6.11 (mean $\pm$ SD, N=31) (Fig. 5b). The transition distance was smaller than the maximum distance, indicating that transitions to similar patterns were common. Time domains for consequent appearance of similar patterns were frequently observed suggested a possibility that the hierarchical information processing is performed in which the consecutive patterns constitute a new pattern.

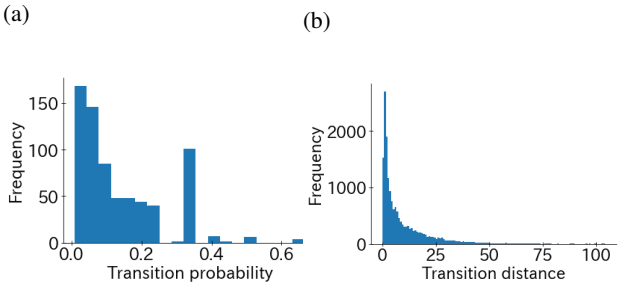

Fig. 5: (a) An example of the distribution of the state transition probabilities between cell assemblies classes. (b) An example of the distribution of the transition distances (E18DIV64).

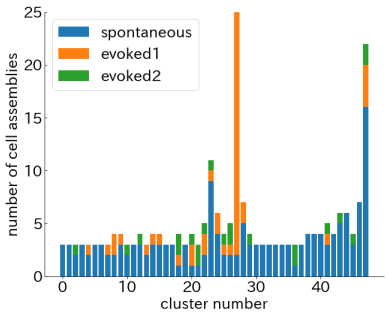

Fig. 6: An example of the number of spontaneous and evoked cell assemblies in each cluster (E18DIV71).

D. Similarity between Spontaneous Activity and Evoked Responses

The cell assemblies obtained from spontaneous responses and those obtained from evoked responses were classified. The evoked activity was defined as the activity within 100 ms after the stimulation. As a result, there were clusters that included both spontaneous responses and evoked responses (Fig. 6). The result indicates that spontaneous activities and evoked responses are not completely independent, but are share resemble activities.

IV. CONCLUSION

We divided cell assemblies into groups consist of exceedingly similar cell assemblies by the indiscernibility-based clustering, called "rough clustering", and analyzed their characteristic. As the result, certain classes of patterns with few different components (active electrode channels) were extracted in cell assembly classes. In addition, we analyzed activities as the stochastic process whose state space is the cell assemblies classes. The transition distance was smaller than the maximum distance, indicating that transition to similar patterns was common. Time domains for the consequent appearance of similar patterns were observed, suggested the hierarchical information processing is performed in the cultured neurons.

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