# The Stability and Periodicity of neuronal network activity pattern repertory.


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## ABSTRACT

The dissociated rat hippocampal neurons on a multi-electrodes array dish are useful as simple model of brain information processing system. We analyzed spontaneous activity in the living neuronal network to investigate periodicity and stability of neuronal network activity. Electrical activity pattern at 5 ms time window was represented as a feature vector with 64 elements 0 or 1, corresponding to presence or absence of spike detected at each electrode. X-means clustering method with kkz algorithm preprocessing was applied to the feature vector of each time window. The number of clusters was stable for 30 min with some fluctuations. As extending of clustering range from 5 min to 30 min in 5 min increments, the estimated number of cluster increased, suggesting the number of activity patterns was not stable and increase. However, highly reproducible clusters were stable against extension of clustering range. In addition, the number of highly reproducible clusters was saturated at approximately for 40 s clustering range. These results suggested that the spike patterns compose limited number of highly reproducible clusters and a lot of small clusters derived from reproducible clusters, and highly reproducible clusters were expressed repeatedly. Semi-artificial neuronal network possessed pattern repertories and they are considered to be able to express certain states.


## General Terms

Algorithms, Measurement


## Keywords

X-means, dissociated culture, extracellular potential multi-site recording system, pattern repertory, spontaneous neuronal activity.


## 1. INTRODUCTION

Higher brain function is performed by dynamics of a complex neuronal network in a brain system. To elucidate the dynamics in self-organized we focused on the interaction between neurons in the network. A small-scaled, cultured neuronal network possessing fundamental brain function is a suitable experimental material (Fig.1). The electrical patterns of the living neuronal network generated depending on the functional connectivity. The functional connections between neurons are the substances of cell assemblies, proposed by D.O. Hebb, in which neurons cooperatively behave with the flexible functional synaptic connection [1]. In a rat hippocampal dissociated neurons, the distribution of the dynamical functional connections is not uniform and there are some hubs with a lot of inputs are composed autonomously [2]. Although the same network structure is physically formed, activity pattern in the network is not always uniform. The various activity patterns are observed, which are quickly updated by switching the functional connections. The existence of dynamic cell assembly means that neuronal network is not static but dependent on dynamical state. Recently, the analysis of the network dynamics in a dissociated culture is advanced by some groups [3-5].

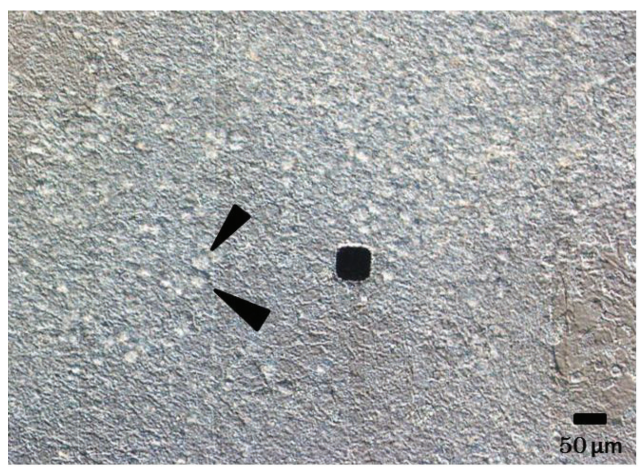


Fig.1 Rat hippocampal dissociated culture (E18DIV20).

The arrow indicates the cell position.

The spatiotemporal pattern of the spike activity evoked by an electrical input was almost reproducible. The patterns of responses evoked by the different inputs do not resemble each other, suggesting that the living neuronal network possesses ability to express several patterns independently. Thus, cultured neuronal network may operate electrical patterns as symbols corresponding to external objects. However, the response after the stimulation is a result of integration of evoked activity and internal state of the network, driven by spontaneous activity. Spontaneous electrical activity frequently was observed in a



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cultured neuronal network approximately a week after starting the culture. Spontaneous activity is separated from external phenomenon, thus it has been often regarded as only a noise for sensory organ inputs. However, there are some possibilities that spontaneous activity influence on information processing in a brain [6-8] and spontaneous activity also contributes to information processing in a brain. It is reported that certain patterns of spontaneous activity were expressed repeatedly at the stable cycle in acute slice preparation [9]. So the evoked response has complex fluctuations, and it is the one of the origin of complexity of the network dynamics in the cultured neuronal network. We have the hypothesis that the internal state consisted by spontaneous activity is regulates and maintains by spontaneous activity. Therefore, it is critical to describe the characteristics of spontaneous activity, which is not completely periodic and reproducible. In this report, we proposed the method to estimate periodicity of spontaneous activity using clustering method. In this method, neuronal activity pattern was classified from the viewpoint of similarity and periodicity of the pattern.

# 2. MATERIAL AND METHOD

## 2.1 Rat Hippocampal Culture

Rat hippocampal neuronal cells were cultured in previously reported method [2]. Embryonic day 18 (E18) Wistar rat hippocampal neurons were cultured on the multielectrodes array dish (MED probe, Alpha MED Scientific, Japan) [10]. The hippocampal region was cut off from cerebral cortex. Neuronal cells were dissociated by 0.175% trypsin (Invitrogen-Gibco, USA) in $Ca^{2+}$ and $Mg^{2+}$-free phosphate buffered saline (PBS-) supplemented with 10 mM glucose at 37 °C for 10 min. The glass ring (cloning ring, inner diameter of 7 mm) was put on the center of a MED probe. $3{\times}10^5$ neurons were seeded inside the ring to avoid sticking of cells to reference electrodes located at marginal culture area. In this case, the cell density was 7800 cells/$mm^2$. Culture medium was mixture of Dulbecco’s modified Eagle’s medium and F12 medium (DMEM-F12) containing 5% horse serum (Invitrogen-Gibco, USA), 5% fetal bovine serum (Invitrogen-Gibco, USA), 100 U / 100 μg/ml Penicillin-Streptomycin (Invitrogen-Gibco, USA), and 5μg/ml insulin (Sigma-Aldrich, USA). Neuronal cells are incubated in a $CO^2$ incubator at 37°C temperature with 5% CO2. All the procedure of animal experiments was conducted according to the “Kwansei Gakuin University Regulations for Animal Experiment”.

## 2.2 Measurement of extra cellar potentials

Electrical activity of living neuronal network was measured using the extracellular potential multi-site recording system (MED64, Alpha MED Scientific [10]). All experiments performed in room temperature. Culture was incubated for 10 min before measurement preventing influences by the drastic change of the temperature of culture medium. In this study, the embryonic day of cultured neurons was 18. The days in vitro (DIV) of the cultures used for electrophysiological experiment were 20 – 45 days. Electrical signals of action potentials measured by an electrodes-array on MED probe were amplified 1000 fold, were applied A/D conversion, and were stored at the hard disk of a PC/AT compatible computer. The A/D conversion was performed at 20 kHz sampling frequency and 16 bits quantum bits. Softwares for recording were MED64 Conductor (Alpha MED Scientific) and Spike Recorder (SPR), which developed in our laboratory.

## 2.3 Analysis of spatiotemporal patterns of spontaneous activity

LabVIEW and C# language were used for describe programs for analysis of spatiotemporal spike pattern. SPR was used for measurement electrical signals and spike counts. SPR counts the spikes of action potentials at each electrode within the 5 ms time window. Numbers of spikes within 5 ms time windows were counted and thus spike numbers were necessarily 0 or 1, because the width of time window allows at most single spike. Electrical activity pattern at a certain time window was represented as a feature vector each of which 64 elements was 0 or 1, corresponding to presence or absence of spike detected at each electrodes. Previous to the clustering, principal component analysis was applied to the feature vector of network activity for dimensional compression, to avoid “curse of dimensionality”. Then X-means clustering method with kkz algorithm preprocessing was applied to spike rate data recorded for 30 min [11]. Kkz preprocessing algorithm determines initial seeds for cluster center (Fig.2) [12]. In X-means algorithm, recursive 2-means is repeated until Bayesian Information Criterion (BIC) no more changes. The method generates more stable clusters than K-means, and is resistant to hyper fractionation. In addition, X-means method does not require for prior determination of the ideal number of clusters. The number of clusters is automatically estimated by X-means clustering. This feature is appropriate for analysis of spontaneous activity, because the number of ideal clusters of spontaneous activity is unknown. In addition, we analyze the reproducibility of each cluster.

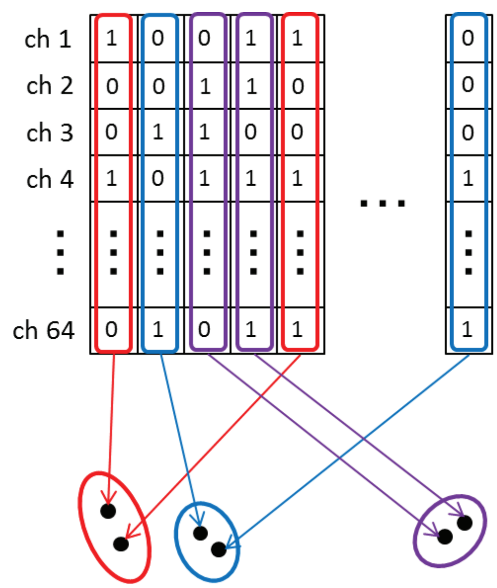


Fig.2 Feature vectors and clustering method

# 3. RESULT AND DISCUSSION

## 3.1 Transition of the number of cluster

The number of clusters of spontaneous activity was estimated. Clustering was applied to the part of recorded data with various time lengths. The number of clusters was tends to increase with extending the clustering range (Fig.3). The number of clusters were 331.40±31.81, 708.20±76.16, 596.40±45.22, 770.40±117.71, 911.00±77.04 and 1105.60±57.76 (mean ± SE) for 5 min, 10 min, 15min, 20 min, 25 min and 30 min, respectively. It is because dates have variation, there is little drop between 10 min to 15 min. However, tendency for the number of clusters increases were observed. The data suggest that novel spatiotemporal patterns of activity emerged during whole recording time of 30 min, and not all the new patterns included in the existing clusters. However, 0-1 feature vectors equally resemble each other, thus the clustering results were too sensitive for the differences among data.

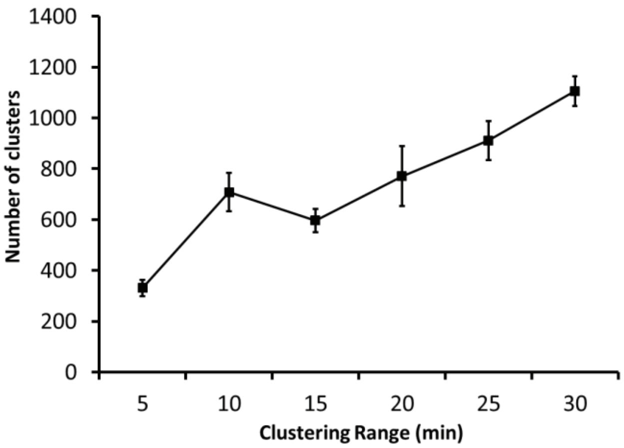


Fig.3 Transition of the number of clusters in each time for analysis (N=5). Error bar is standard error.

## 3.2 Arrival time to 90% of the appearance of the clusters

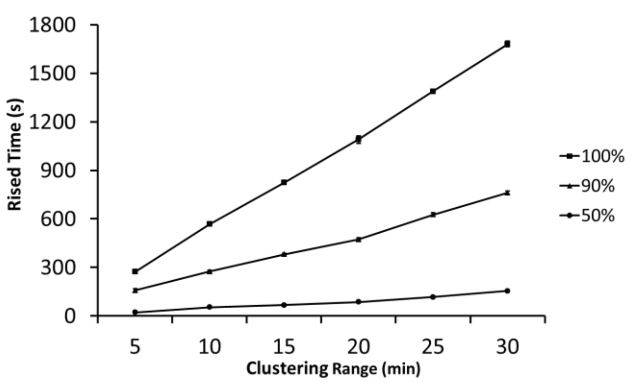


Fig.4 Rised time of 50%, 90% and 100% (N=5).

Error bar is standard error.

We calculated the raised time to 50%, 90% and 100% of the total number of clusters. In addition, to focused on the time to reach 90% (Fig 4). Time to reach 90% at any time of analysis, we found that less than half of the time is to be analyzed. From this result, it suggested that the period of spatiotemporal pattern repertory of neuronal network ware short time.in addition, the cluster of high incidence ware appear fast stage and appear repeatedly. In contrast, it suggested that cluster of low incidence ware appear randomly and noise.

## 3.3 The reproducibility of clusters

The reproducibility of the cluster was estimated. We calculated the ratio of the number of feature vectors belonging to a certain cluster to the total number of the feature vectors during clustering time and defined the ratio as the reproducibility of the cluster (Fig.5). Only approximately 15 clusters repeatedly appeared in all analyzed clustering ranges. These clusters correspond to the clusters with over 1% reproducibility. The number of clusters with over 1% reproducibility was stable for clustering ranges (Fig.6). These clusters ware the pattern repertory of spontaneous electrical activity in the neuronal network. The reproducibility distribution indicated that the clusters with over 1% reproducibility were stable within 5 min clustering range. Therefore, we analyzed the number of clusters in shorter clustering range (Fig.7).

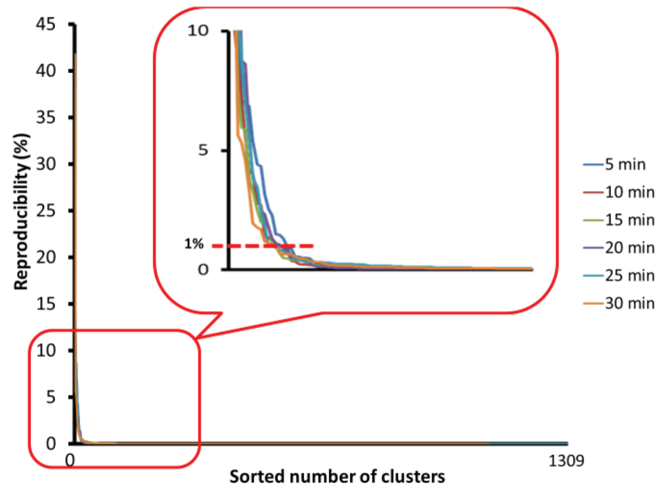


Fig.5 Reproducibility graph of each time for analysis.

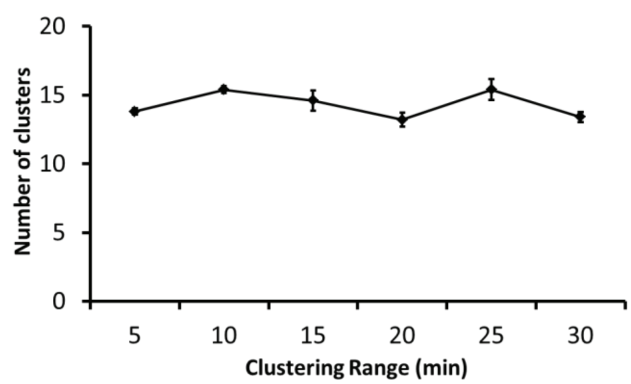


Fig.6 Number of clusters with over 1% reproducibility (N=5).

Error bar is standard error.

In the analysis of the data less than 1 min, the clusters with higher reproducibility ware increase gradually and became to be stable state in approximately 40 s. The number of major clusters was stable during over 40 s, and did not increased if clustering range was wide enough. In the case that clustering range was longer than a cycle of periodic patterns, the number of clusters does not increase accompanying the extension of the clustering range. It is considered that the emerging firing pattern repertories are limited in certain number and firing pattern was periodic, among only major repeated clusters.

The experimental results suggest that the repertoires of the spatiotemporal pattern of spontaneous electrical activity are periodic, and we can roughly estimate the cycle of pattern repertoires, which is close to clustering range at saturation of number of major repeated clusters.

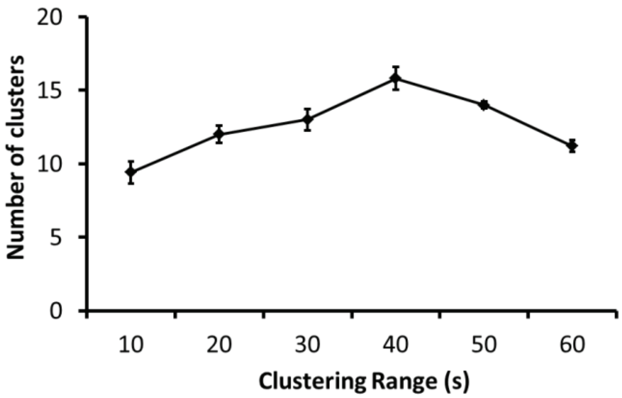


Fig.7 Number of cluster that 1% over incidence of analysis of the data less than 1 min (N=5). Error bar is standard error.

## 4. CONCLUSION

The number of clusters was stable for 30 min with some fluctuations. The estimated number of clusters increased as extending time of clustering range. However, just for highly reproducible clusters were stable for the extension of clustering range. In addition, the number of highly reproducible clusters was saturated at approximately for 40 s clustering range. These results suggested that the repeated spike patterns compose limited number of highly reproducible clusters and a lot of small clusters derived from reproducible clusters. Semi-artificial neuronal network possessed pattern repertories and they are considered to be able to express certain states.

## 5. ACKNOWLEDGMENTS


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