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Analysis of neuronal cells of dissociated primary culture on high-density CMOS electrode array

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Abstract— **Spontaneous development of neuronal cells was recorded around 4–34 days *in vitro* (DIV) with high-density CMOS array, which enables detailed study of the spatio-temporal activity of neuronal culture. We used the CMOS array to characterize the evolution of the inter-spike interval (ISI) distribution from putative single neurons, and estimate the network structure based on transfer entropy analysis, where each node corresponds to a single neuron. We observed that the ISI distributions gradually obeyed the power law with maturation of the network. The amount of information transferred between neurons increased at the early stage of development, but decreased as the network matured. These results suggest that both ISI and transfer entropy were very useful for characterizing the dynamic development of cultured neural cells over a few weeks.**

I. Introduction

There are many techniques for training artificial neural networks to optimize an explicit objective function, e.g., back propagation, genetic algorithm, and Q-learning [3], [5], [10]. But with biological systems in general, there are no explicit functions that need to be optimized. Indeed, a characteristic trait of a biological system is its autonomy, which is found in the spontaneous primitive movements of a bacterium, and even in the sophisticated learning skills and action selection of human beings. Our main interest is the study of developmental processes and of the emergent learning capabilities expressed by a biological neural system. As a first step, we focused on the development of cultured neural cells.

Biological neural cells cultured *in vitro* show development, aging, and spontaneous activities, which are rarely studied in computational neural cells. These features of biological cells are the focus of the present research. More specifically, we aim to reveal the underlying dynamics of the developmental processes.

By recording the development of cultured biological neural cells on a CMOS (complementary meta-oxide-semiconductor) array glass plate, we monitored the temporal evolution of electrical signals over a few weeks. From direct observations, we noticed that the activities of neural cells were rather independent in the early stage, after which spontaneous synchronized activity emerges; other quasi-stable states seem to appear in the later stage.

Biological neuronal cells have been extensively characterized using conventional microelectrode array (MEA) [1], [7], [8]. However, such characterization is not precise because the locations of recording sites in MEAs are predetermined, with an inter-electrode distance of 200 μm, and thus neurons far from these recording sites are not included. Alternatively, optical imaging can be used to study the Ca^{++} dynamics of any neuron of interest; however, the temporal resolution is not high enough to characterize the action potentials of each neuron.

High-density CMOS array is an emerging instrument for investigation of the spatio-temporal activity of neuronal culture cells in detail. The array has 11,000 recording sites with an inter-electrode distance of 17 μm, i.e., in the order of cell body size, and a sampling rate of over 20 kHz. This high spatio-temporal resolution allows precise recording of action potentials from the identified cell bodies of neurons. In the present work, we use the CMOS array to characterize inter-spike interval (ISI) distributions from putative single neurons, and estimate network structure based on transfer entropy, where each node corresponds to a single neuron.

The paper is organized as follows. In Section II, the specifications of the CMOS array and the biological conditions for neural cells are provided. The method for cultivating cells and some associated techniques are also described. In Section III, recorded developmental processes in cultured plates will be examined by the method of transfer entropy. In addition, we will validate the method of transfer entropy by using a computer simulation of a neural model. Finally, in Section IV, the paper is summarized and future problems are discussed.

II. Signal recording of neural spikes and ISI distribution

A. In vitro experimental settings

To measure the electrical activity of cultured neurons, we employed a high-density CMOS microelectrode array [2]. This device is useful for assessing extracellular electrophysiological activity with a high spatial resolution, at a concentration of 11,011 electrodes over an area of about 4 mm^2. Using this high spatial resolution, we localized neuronal somata and recorded their activities.

1) Dissociated neuronal culture: All procedures were approved by our institutional committee at the University of Tokyo, and were performed in accordance with "Guiding

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Principles for the Care and Use of Animals in the Field of Physiological Science" of the Japanese Physiological Society. The neuronal cultures were prepared from the cerebral cortex of E18 (embryonic 18 day old) Wistar rats. The cortex was triturated with trypsin and dissociated cells were plated and cultured on high-density CMOS microelectrode arrays coated with polyethylenimine and laminin. For the first 24 h, cells were cultured in neurobasal medium containing 10% horse serum, 0.5 mM GlutaMAX and 2% B-27 supplement. After the first 24 h, half of the medium was replaced with growth medium in the form of Dulbecco's modified Eagle's medium with 10% horse serum, 0.5 mM GlutaMAX and 1 mM sodium pyruvate. During cell culturing, half of the medium was replaced once a week with this growth medium. These cultures were placed in an incubator at 37 °C with an H_2O-saturated atmosphere consisting of 95% air and 5% CO_2. The number of plated cells on CMOS array chips was 35,000 on Chip#1 and 14,000 each on Chip#2 and Chip#3.

2) Recording of neuronal activities: Recording of neuronal activities was performed with high-density CMOS microelectrode arrays. Before recording the activities of neuronal somata, almost all of the 11,011 electrodes were scanned to obtain an electrical activity map with which we estimated the locations of the somata. A scan session consisted of 95 recordings; each recording was conducted for 60 s with about 110 electrodes that were selected randomly, while avoiding overlap with already selected electrodes. An electrical activity map was obtained from the scanned data by calculating the average height of the spikes for every electrode. We assumed that the neuronal somata were in the vicinity of the local peaks in the Gaussian-filtered electrical activity map. About 100 of the higher level peaks are selected, and then the nearest electrodes were selected for the recording. If the number of local peaks was fewer than 126, all the peaks were selected. The electrical activities were recorded for 30 min from these selected electrodes. All recordings were done at a 20-kHz sampling rate using the LimAda spike detection algorithm [9] with a threshold of 5. Unexpected double-detected spikes were removed from the data before analysis.

B. ISI distribution of neurons showing power law / exponential decay

The recording times for each chip were 7–18 days *in vitro* (DIV) for Chip#1, 6–34 DIV for Chip#2, and 4–24 DIV for Chip#3. We used the data during these periods for the analyses. First, we analyzed the ISI distribution of neural activities. Fig. 1 shows examples of ISI distributions computed from two channels. In the earlier stage of culture, most of the channels seemed to exhibit exponential decay (Fig. 1(a)), while the channels shown in Fig. 1(b) and (c), which represent a later stage of culture, tended to obey the power law. To quantify this tendency, we plotted the frequency of ISIs on a logarithmic scale. If the ISI distribution follows the power law, it should be a straight line. The degree of fitting is measured by the R-squared value, which can be considered an index of fitting the power law. Fig. 2 shows the R-squared values for each sample (chip), which indicate that the R-squared values as the number of DIV increases.

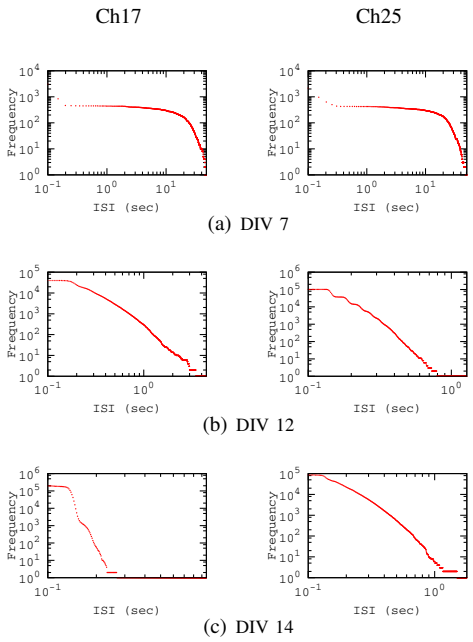


Fig. 1. Examples of ISI distribution of neural activities recorded in the 17^{th} and 25^{th} channels on Chip#1. The X and Y axes depict the logarithm of ISI and frequency respectively. (a), (b) and (c) display the results for 7, 12, and 14 DIV respectively. Estimated exponents for the respective channels (17^{th} and 25^{th}) are as follows: (a) -1.28(0.59) and -1.40(0.57); (b) -3.50(0.98) and -5.44(0.95); (c) -3.77(0.57) and -4.76(0.98). The values inside parenthesis denote the R-squared values of the regression lines.

III. Reconstruction of Neural Structure by Transfer Entropy

In this section, we used transfer entropy to estimate the synaptic connectivity of the cultured neurons. Transfer entropy measures directed information transfer [6]. For instance, a higher transfer entropy from one neuron to another indicates that the first neuron affects the second strongly. Therefore, transfer entropy enables to find out the effective synaptic connectivity. We first define transfer entropy, and then apply it to artificial neural networks in order to obtain the optimal parameter set for the transfer entropy to estimate the network topology. Using those parameters, we further apply transfer entropy to cultured neural cells to infer their topology.

Transfer entropy from a time series I to another time series J is calculated as follows:

$$T_{I\to J} = \sum_{n=0}^{N-1} p(i_{n+1}, i_n^{(k)}, j_n^{(l)}) \log \frac{p(i_{n+1}|i_n^{(k)}, j_n^{(l)})}{p(i_{n+1}|i_n^{(k)})} \qquad (1)$$

where i_{n+1} denotes the variable at the $(n+1)$th step of I. k and l are the number of past variables in I and J, where $i_n^{(k)} = \{i_n, i_{n-1}, \ldots i_{n-k+1}\}$ and $j_n^{(l)} = \{j_n, j_{n-1}, \ldots j_{n-k+1}\}$. When i_{n+1} is conditioned by $j_n^{(l)}$, $p(i_{n+1}|i_n^{(k)}, j_n^{(l)})$ takes a higher value than $p(i_{n+1}|i_n^{(k)})$, while, if i_{n+1} is independently determined from $j_n^{(l)}$, then the two components take the same value.

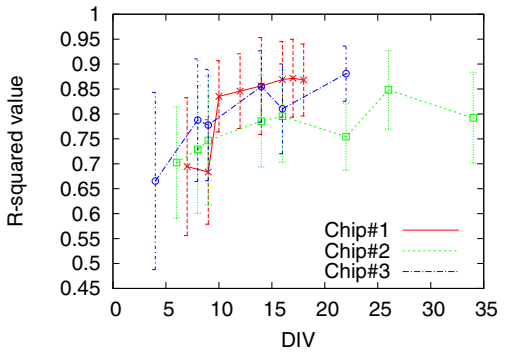


Fig. 2. Change in the R-squared values with the number of DIV. The R-squared value is obtained from a regression line to fit to ISI distribution, which is plotted on a logarithmic scale. When the R-squared value is closer to 1.0, the ISI distribution is more likely to obey the power law.

A. Testing artificial neural networks

Transfer entropy analysis was first applied to a computational neural model. The model is built around Izhikevich neurons connected through artificial synapses [4]. The Izhikevich neurons form a simple model of cortical neurons that is implemented by a system of two differential equations modeling the membrane potential and the refractory period. When the membrane potential reaches a threshold value (for instance, 30 mV), a spike is emitted. This spike will be transferred to post-synaptic neurons through some shared synapses. The voltage on arrival is the original spike strength, modulated by the efficacy of the synapses. For instance, an initial spike of 30 mV traveling on a synapse with an efficacy of 0.5 will deliver a voltage of 15 mV to the post-synaptic neuron. Every synapse has a delay of 1 ms between the time of emission and the arrival of a spike.

The complete model is composed of seven neurons: four input neurons receiving randomly generated external stimulations, two internal neurons and one output neuron. The parameters for the Izhikevich neurons correspond to the regular spiking model($a = 0.02$, $b = 0.2$, $c = -65mV$ and $d = 6$). Different types of connectivity patterns have been tested, ranging from fully interconnected to sparse (Fig. 3 (a)–(c)). The strength of the connection is randomly assigned based on uniform distribution. Every update of the model represents a 0.1-ms step in time, which ensures its mathematical stability. The total duration of a test is 1000 s, which corresponds to 10,000,000 updates of the model.

B. Reconstruction of artificial neural networks

From the time series of neural activities obtained from the computer simulation, we calculated the transfer entropy between neurons. Depending on the transfer entropy, we estimated the network structure of the artificial neurons. A synaptic connection from one to the other was assumed when the transfer entropy between two neurons was higher than the threshold. Then, we compared the topology of the reconstructed network and the original network displayed in Fig. 3 (a)–(c). The amount of false edges for each topology is shown in Fig. 4. Δt in this figure represents the bin-length

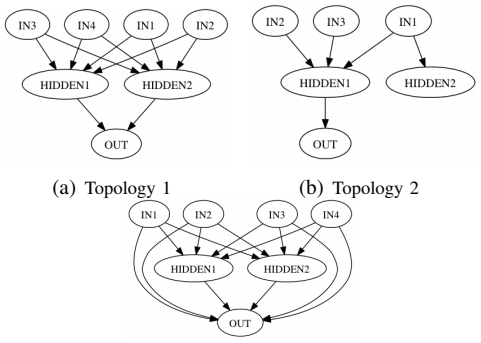


Fig. 3. Topology of the original network used in the computer simulation.

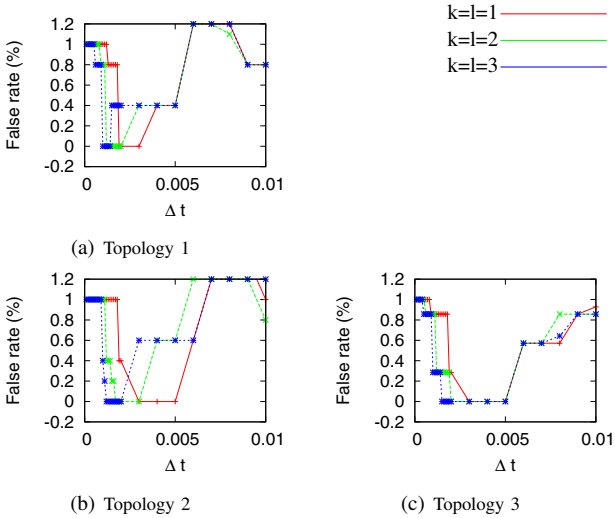
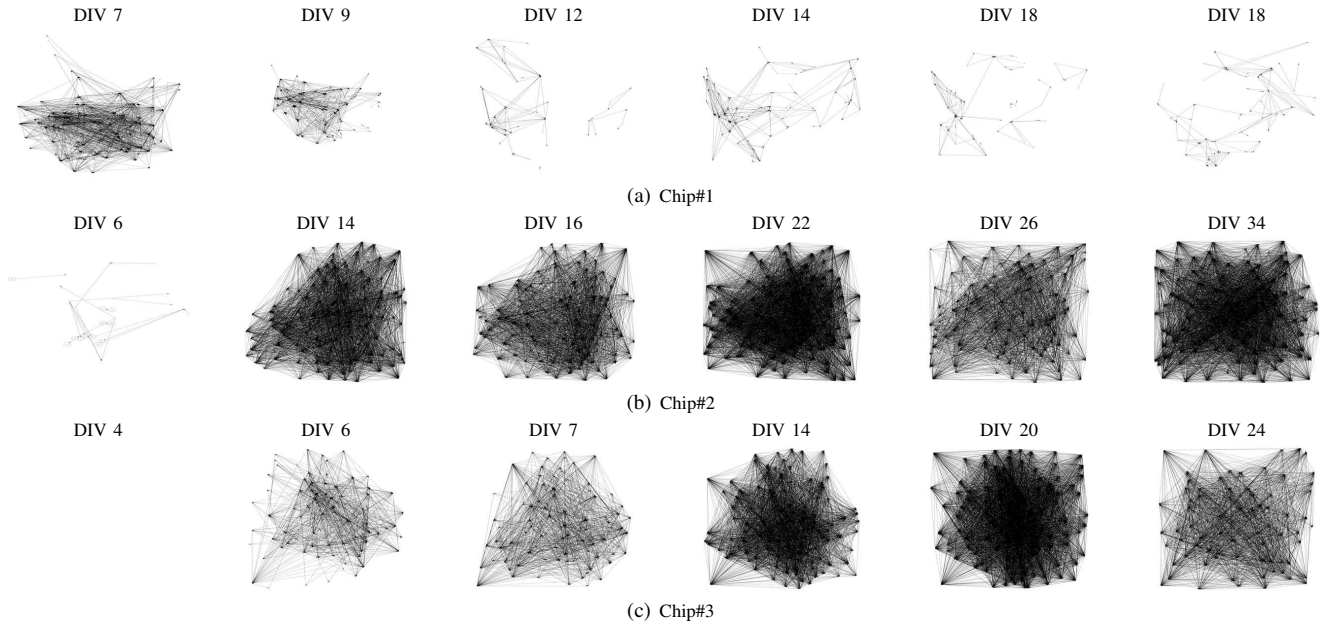


Fig. 4. Number of false connections with different Δt values. The threshold was set to 0.005.

of the compressed time series, in which the spike data are renormalized into bins of length Δt. This figure shows that there are optimal regions for reconstruction of the original topology; $\Delta t = 0.002$ s, $k = l = 2$. These optimal regions were used to estimate the connectivity of the cultured neural cells.

C. Reconstruction of cultured networks

Fig. 5 shows the estimated network structure of neurons. Edges are drawn if the transfer entropy from one neuron to the other is higher than the threshold, which is set to 0.00001 in Fig. 5. It indicates that the connectivity pattern changes following the growth of neurons. From Fig. 5, we computed the evolution of the number of edges, which is shown in Fig. 6. In the earlier stages, the number of edges seems small, but it increases afterward. This suggests that the amount of information transferred increases with growth of the network; that is, more information is mutually transferred among neurons. However, in the case of Chip#1, the number of edges decreases after 9 DIV, which implies that the amount of transferred information between neurons increased at the early stage of development, but decreased as the network become mature.



(a) Chip#1

(b) Chip#2

(c) Chip#3

Fig. 5. Examples of network structure reconstructed by transfer entropy.

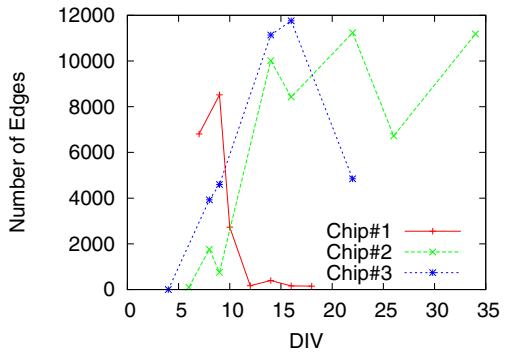


Fig. 6. Number of edges in the reconstructed network as determined by transfer entropy.

In comparison with the result shown in Fig. 2, the number of edges peaks at 9 DIV, while the R-squared value of Chip#1 shows a large increase at 10 DIV. The result for Chip#2 shows that the number of edges is maximum around 15 DIV, when the R-squared value reaches a plateau. Therefore, power law behavior and the efficiency of information transfer seem to be related. In the future, we will conduct experiments with more neuron plates to confirm check this tendency.

IV. Conclusions

We characterized the neuronal activities of putative single neurons by their primary dissociated culture on CMOS array. With maturation of the network, ISI distributions gradually showed a tendency to obey the power law. On the other hand, the amount of information transferred between neurons increased at the early stage of development, but decreased as the network became mature. These results suggest that both ISI and transfer entropy well characterize the dynamic development of cultured neural cells over a few weeks. We plan to investigate this tendency further by using more chips and longer recording periods.

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