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Interaction and Intelligence in Living Neuronal Networks Connected to Moving Robot

Suguru N. Kudoh[1,2] Takahisa Taguchi[1], and Isao Hayashi[3]
1. RICE, AIST, Ikeda, Osaka, Japan 2. Interactions and intelligence, PREST, JST, Tokyo, Japan
3. Faculty of Informatics, Kansai University, Takatsuki, Osaka, Japan

***Abstract*—The temporal patterns of spontaneous action potentials are analyzed, using the multi-site recording system for extracellular potentials of neurons and the living neuronal networks cultured on a 2-dimensional electrode arrays. We carried out the system integration for Khepera II robot and living neuronal network. We call the system as "biomodeling system". Our goal is reconstruction of the neuronal network, which can process "thinking" in the dissociated culture system.**

I. INTRODUCTION

Neurons form complex networks and it seems that the living neuronal network can perform a certain type of information processing [1]-[3]. We are interested in intelligence which is autonomously formed in vitro. Dissociated culture system with multi-electrode array is fully useful for elucidation of network dynamics of neurons. The system allows us to examine the process for the information processing by analyzing the behavior of neurons in a cellular level and a molecular level. Dissociated neurons begin to elongate neurites on multi-electrode array and reconstruct complicated living neuronal networks (Fig.1). We investigate the spatio-temporal pattern of spontaneous action potentials. Functional connectivity between all the combinations of neurons were estimated and indicated simultaneously in 2-D map. This "Connection map" revealed that hub-like neurons with many functional connections were often observed in all distinct cultured networks, suggesting that the network structure were not random and that coupled functional neuron assemblies were autonomously formed. In addition, the functional connection between neurons changed drastically after the induction of synaptic potentiation, and additional hub neurons newly emerged. Thus, spontaneous activities are enough to construct dynamic functional assemblies of neurons even in a cultured living neuronal networks and synaptic potentiation can induce re-organization of such assemblies of neurons. Although their environmental conditions are different from in brain organs, it seems that the dissociated neurons are equipped with the critical, fundamental mechanisms for the formation of functional networks. In addition, spontaneous ensemble electrical activities usually observed in enough developed networks, and they are seemed to be able to perform a certain information processing. It means that the living neuronal network can obtain a kind of "intelligence" by interactions with outer world, if it has input and output. According to that concept, we developed integration system of Khepera II robot and living neuronal network, using two kinds of fuzzy logic [4, 5]. We call the system as **"biomodeling system"**. The biomodeling system has a loop procedure, in which the "top-down bio-processing" for sending actuator signals to robot from living neuronal network, and the "bottom-up robot-processing" for electrical stimulation to living neuronal network from robot are connected for interaction between neuronal network and robot. We are here analyzing the interaction between the robot and neurons, and discuss reconstruction of the neuronal network, which can process "thinking" in the dissociated culture system.

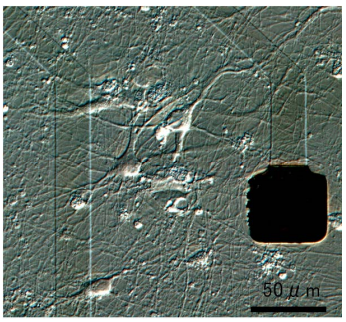

Fig. 1 Cultured neuronal network on multielectrode array. Neurons re-constructed complex networks in vitro. The black squares are electrodes. Black bar indicates 50 μm.

II. METHODS FOR BIOLOGICAL EXPERIMENTS

A. Neuron culture

The conduct of all experimental procedures was governed by The Animal Welfare, Care and Use Committee in AIST. The hippocampus neurons were prepared from a Wister rat on embryonic day 17-18 (E17-18) and cultured by the previously described method [6]. Briefly, neurons were dissociated by treatment with 0.175% trypsin (Gibco, U.S.A.) and cultured by plating 500,000 cells in a 7mm diameter-glass ring on poly-D-lysine coated MED probe (Alpha MED Sciences, Japan), which has 64 planar placed microelectrodes. The medium is based on D-MEM/F12, supplemented with 5% horse serum (Gibco, U.S.A.) and 5% fetal calf serum (Gibco, U.S.A.).

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B. Spike recording by multi-electrode array

The field action potentials were recorded 10-100days after the start of the culture. The spontaneous action potentials (sAPs) were gathered with the MED64 system (Alpha MED Sciences, Japan) [7] at a 10-20 kHz sampling rate. Evoked field action potentials (eAPs) at 62 sites in the cultured networks were recorded with the MED64 system at a 20 kHz sampling rate. All experiments were carried out at room temperature (20-25 ^{o}C). The recorded spikes were detected by our developing program, sorted and classified by the amplitude versus decay time distributions using k-means cluster cutting method and converted to event trains.

C. Connection map analysis

All possible combinations of pairs of spike trains recorded by the multi-electrodes were subjected to cross correlation analysis. Then connectivity indices were calculated for each pair. The connectivity index is defined as follows:

$$Con.I. = A_{peak} \times \left(\frac{0.01 \times A_{peak}}{A_{total}} + \frac{1}{\Delta t + 1} + \frac{1}{Kurtosis} \right)$$

where A_{peak} is an area within the 2 msec range around the peak of cross-correlofunction of the pair, A_{total} is the total area of cross-correlofunction, Δt is the distance of the peak of the cross-correlofunction from 0. The connectivity index indicates the robustness of the relationship between the pair. The mean and standard error of all connectivity indices were calculated. When the value of the connectivity index exceeded a criterion, in this case, the mean plus standard error, the pair was assumed to be functionally connected. Each neuron was denoted as a small point in maps, and lines between the points represented the connectivity between the neurons indicated by these points. The color of the lines indicates the relative value of the connectivity index. Functional connections between all combinations of recorded spike trains were depicted simultaneously in this 2-D map. In this study, we generated connection maps from data recorded for 10min, the bin width for calculation of the cross-correlation was 5 msec, and the range of the calculation of cross-correlofunction was 120 msec. Connection maps of serial experiments were expressed as follows. First, connection maps were generated for sequential recordings. Then each point indicating a neural unit in the second recording was shifted to the lower right. The parameters of classified APs, such as mean amplitudes, were compared with the parameters of the initial reference recording, and each point was identified as being the same as an individual unit seen in the initial recording. These units were then depicted at the same position as in connection map of the initial recording, and other units (including newly detected units) were left behind at the shifted positions. Units with more than 20 % fluctuation of the parameters in sequential records were identified as distinct neurons.

III. Drastic re-organization of functional assembry induced by feed-back stimulation

We set up the system in which the living neuronal network interacts to feedback stimulator. In the experiment of Fig.2, only one stimulation pulse was applied to E20 (black square) in the cultured neuronal network when action potentials were detected from both of E48 and E11 (thick frames). We defined this experimental scheme as **"2-detection and 1-stimulation scheme"**. This type of feedback stimulus were started on the 10th day in vitro (DIV10). In the example case of the experiment, these spontaneous electrical activities synchronized highly [8, 9] and the stimulation frequently applied to the cultured network.

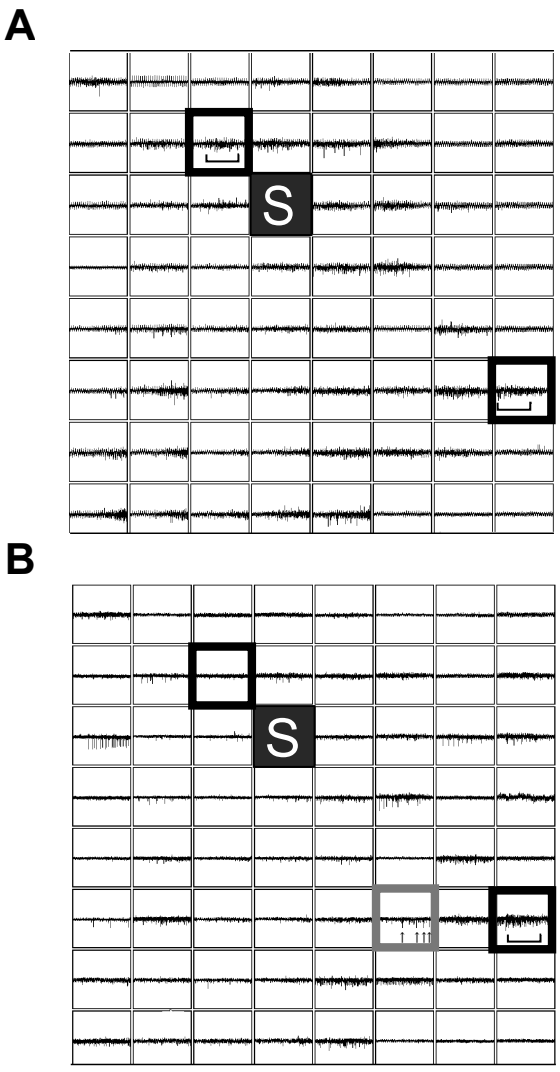

Fig. 2 Drastic change of spatio-temporal pattern of spontaneous action potentials induced by **"2-detection and 1-stimulation scheme"**
A: An example pattern before stimulation. B: A pattern after stimulation.

The stimulation activated the network and evoked next synchronized inputs at the two electrodes. The synchronized inputs triggered next stimulation. Thus, bursting-like

stimulation pattern was generated by the interaction of network and the real-time feedback system (Fig. 3). The important point is that the duration and frequency of this bursting stimulation were determined by the intrinsic state of a living neuronal network. In other words, the living

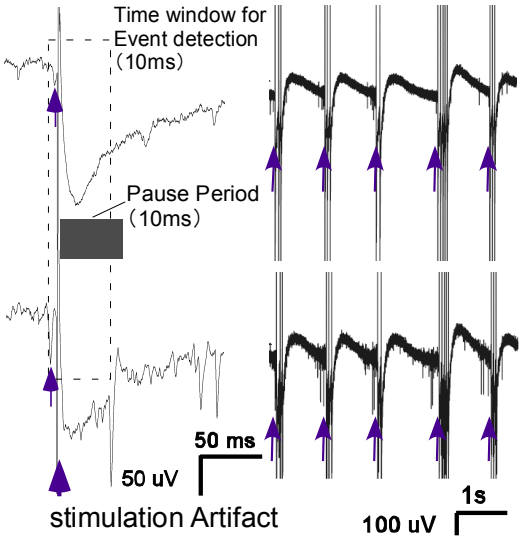

Fig. 3 An example of stimulation pattern. Under "2-detection and 1-stimulation scheme", the real-time stimulation system monitors two electrodes simultaneously and began to apply stimulation to a selected electrode when synchronous inputs detected from these two selected electrodes.

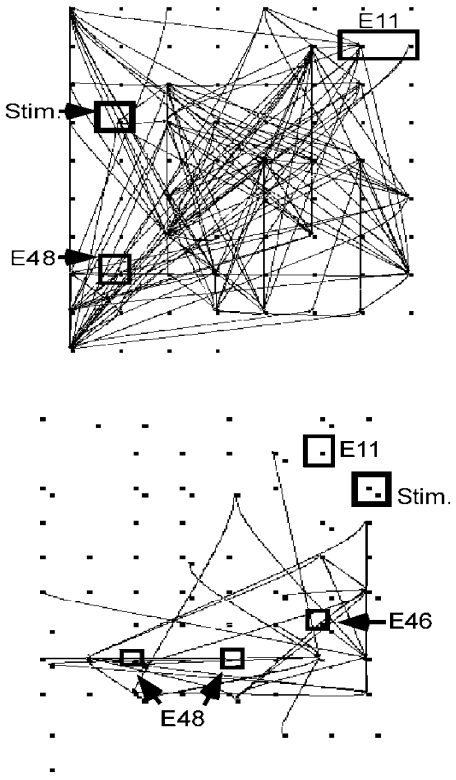

Fig. 4 Connection map before (upper panel) and after (lower panel) feedback stimulation.

neuronal network can control this parameter of the stimulation. Consequently, even after the 24hr feedback stimulation, drastic change of activity pattern occurred. Interestingly, a particular neuron recorded from one of monitored electrodes reduced its synchronous spontaneous activity, while another increased its activity. Connection map analysis revealed that after "2-detection and 1-stimulation scheme" feedback stimulation caused suppression of spontaneous electrical activities in the network and induced drastic re-organization of functional connections between neurons, when these activities are initially almost synchronized (Fig. 4).

Real-time feedback stimulation reduced synchronized activities in one of monitored electrodes. This result is still preliminary stage, but it certainly suggests that living neuronal network can re-organize their activity pattern depending on environmental I/O interaction. In other words, the cultured living neuronal network can embed "environmental pattern" into their functional connections. We should perform control experiments, in which the stimulation is not contingent on activity and verify whether the closed-loop system is responsible for the observed drastic changes. In addition, we have to confirm whether the same phenomenon happens in various connections in complex neuronal networks.

IV. INTERACTION TO OUTER WORLD OF LIVING NEURONAL NETWORKS INTERFACED WITH MOVING ROBOT

In addition, we are developing the system in which living neuronal network interacts to outer world by the intermediary of miniature moving robot. We use Khepera II robot (K-Team), Lab View (National instruments) because Khepera II can be programmed easily by LabView environment and MED64 system uses DAQ board of National instruments (Fig. 5). The Hybrot (Hybrid living + robotic) idea was previously reported by Potter's group [10, 11]. We extended the idea and provided a program which generates "premised control rules" for making a robot avoid obstacles, instead of making the living neuronal network generate such rules autonomously. We think that it is impossible for living neuronal network to autonomously generate process for meaningful information processing. Creatures are also impossible to get such algorithms without emotional system, sense of pain, and so on. These basic systems offer "meaning" from the point of survival. The value of the behavior is assessed on the basis of whether the behavior is suitable for survival or not.

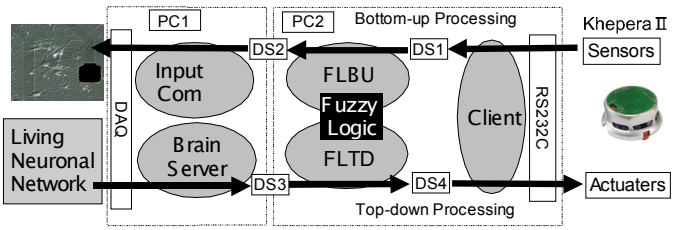

Fig. 5 Miniature moving robot KheperaII connected to living neuronal network.

Robot with living neuronal network has two Fuzzy Control Unit, one of them is for control of the actuators of the robot, FLTD (Fuzzy Logic in Top-Down Process), in the top-down bio-processing, and another is for control of the electrical stimulation to the neuronal network, FLBU (Fuzzy Logic in Bottom-Up Process), in the bottom-up robot-processing (Fig. 6). Robot control unit receives 8 inputs from the neuronal network, and each input is the number of action potentials detected by 8 adjacent electrodes within 50 msec time window. Stimulation control unit receives outputs of 8 IR sensors of the robot. If obstacles exist near the left side, then the unit applies electrical stimulation to electrode 1, and so on. When Robot control unit detect particular pattern that is evoked by the electrical stimulation to electrode 1, the speed of left actuator is set to be fast. Thus the robot can avoid collision (Fig. 7).

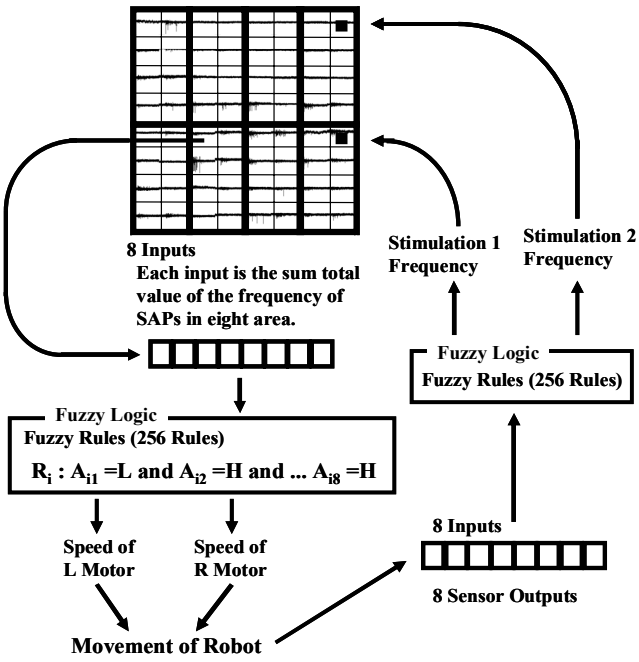

Fig. 6 Fuzzy control of the robot with living neuronal network

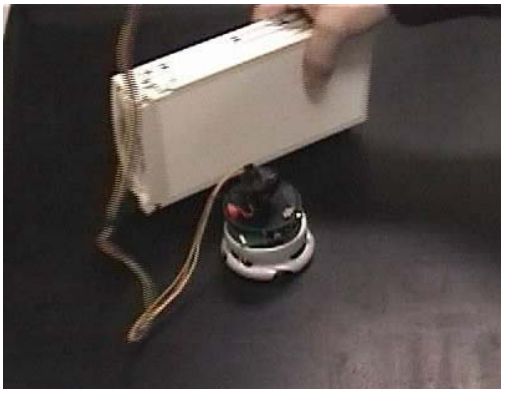

Fig. 7 Collision avoidance of Khepera II robot with living neuronal network (E18D96).

The system consists of 256 fuzzy rules with eight inputs. Each input has two kinds of fuzzy labels of high frequency and low frequency. The 256 fuzzy rules can express all the classified patterns of the action potentials in eight inputs.

Setups of the membership function of fuzzy rules were performed as follows. Maximum value frequency of the action potential in all electrodes was made into the maximum of the horizontal axis of a membership function. The maximum of the membership function assigned to the label of high frequency was at three fourths of the points of the maximum frequency, and maximum of the membership function assigned to a low frequency label was conversely was at one fourth of the points of the maximum frequency.

The method of setting up fuzzy rules is as follows. The differences of the fuzzy number of each rule assigned to bringing an object close to the left side of the robot and to right side were calculated. We focused on the rules with large differences. The rules with large fuzzy numbers for object at left side correspond to particular patterns evoked by inputs related to left IR sensors. In contrast, the rules with large fuzzy numbers for object at right side correspond to particular patterns related to right IR sensors. So that, for example, consequents of the rules for left IR sensor were configured that speed of left actuator is high.

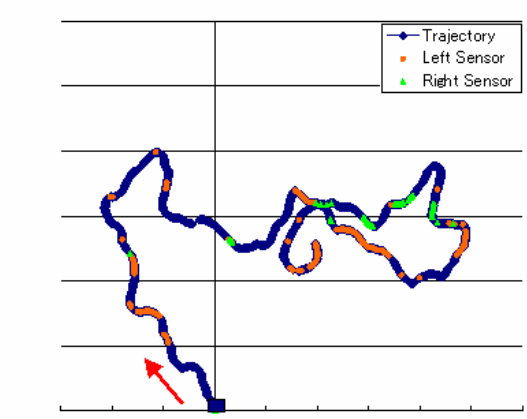

Fig. 8 An example of trajectory of Khepera II robot controlled by cultured neuronal network.

The most important point is that the pattern evoked by electrical stimulation to a particular electrode is not constant. The pattern was varied because the pattern was the result of certain information processing of living neuronal network. So the behavior of the robot was also rather unequable, sometime the robot did not change its way even though there was an obstacle, just like an animal. Fig. 8 shows an example of trajectory of Khepera II robot with cultured neuronal network. Orange dots indicate the sensors located at left side of the robot detect objects. Green dots indicate the right sensors detected objects. Sometimes the robot ignore the sensor inputs, but it feckly avoid collision. In our previous reports [1], the pattern of evoked action potential can be modified by a particular pattern of electrical

stimulation. If we observe adequate modification in the behavior of this robot by interaction to outer world, can we define that phenomenon as the intelligence of living neuronal network? Our ultimate goal is to generate intelligence in culture dish and to observe it.

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