Neurorobot Vitroid
- a living test model for embodiment brain research

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***Abstract*—One of effective approaches to problems of conventional artificial intelligence is "embodied cognitive science". To realize an embodiment of a cultured living neuronal circuit, we provided cultured neurons with a body of a miniature-moving robot. The aim of this neuro-robot system is preparing a testing environment of the suitable relationship between activity of living neuronal network (LNN) and phenomena in outside world. We call our neuro-robot system as "Vitroid", meaning a system as a kind of a "test tube" for cognitive agent made by living component. We have investigated whether the Vitroid with various algorithms for linking neurons and outer world can be embedded a certain a priori behavior, and elucidated the modification of network dynamics induced by robot behaviors. We have developed Vitroid with simplified fuzzy reasoning, and a weighted averaging of selected electrode activity. Both algorithms for linking induced the decrease of standard deviation of the number of electrical events induced by inputs from sensor on the robot. It suggests that the modification of network dynamics is induced by robot behaviors, which is likely in animal brain.**

Keywords-MEA; Dissociated culture; Living neuronal netwrk; miniature-moving robot; closed-loop interaction

I. Introduction

Biological intelligence has characteristic functions suitable for real-world adaptation. Symbol grounding problem, frame problem are often cited as examples of technological difficulties of artificial intelligence reliable in real world. One of effective approaches to these "oft-expressed" problems is "embodied cognitive science"[1,2]. In embodied cognitive science, a body serves suitable relationship between sensors and actuators, which is effective on frame problem by triggering autonomous action to an environmental stimulation. In the concept of "embodiment", some a priori rules for behaviors are embedded in the relationships of sensors and actuators. One of the remaining problems for realization of creature-like intelligence is how to generate "phenomenal consciousness" by artificial components [3].

This philosophical hard problem is difficult to discuss in a field of an experimental science, but we think that we can understand the phenomenal consciousness by constructing such creature-like system by integration of embodied cognitive robotics and self-organizing network of a living neuronal network (LNN) reorganized in vitro (Figure 1)[4,5]. Cultured rat hippocampal neurons reorganize a complex network on a microelectrodes array dish, which enable us to investigate network dynamics by simultaneously measuring field potentials external of multiple neurons. The neuronal circuit expresses spontaneous electrical activity without any external

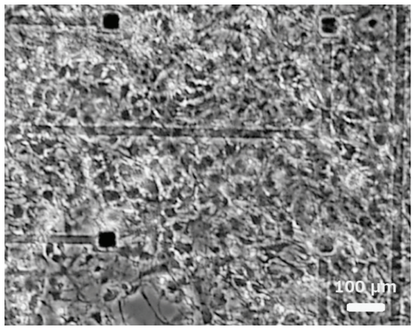

Figure 1. An example of LNN on microelectrodes array dish (E18DIV20). Black squares in microphotograph are planer microelectrodes.

stimulation. It is likely that the spontaneous activity compose certain internal states of the neuronal circuit, fluctuated by interaction of mutually connected neurons. We think that this network dynamics is the critical source of creature-like information processing. The cultured neurons do not have a body, and there is no input and output relationship of cultured neurons and outer world. To realize an embodiment of cultured neuronal circuit, we provided cultured neurons with a body of a miniature-moving robot [6,7]. The aim of this neuro-robot system is preparing a testing environment of the suitable relationship between the network dynamics of cultured neurons and phenomena in outside world. We call our neuro-robot system as "Vitroid" [7], meaning a system as a kind of a "test tube" for cognitive agent made by living component. A central processing unit of Vitroid is a LNN, and all decision-making of the system is performed by LNN. We have investigated whether the Vitroid with various algorithms for linking neurons and outer world can be embedded a certain a priori behavior, and elucidated the modification of network dynamics induced by robot behaviors.

II. Material and Methods

A. Preparing for LNN

Methods for preparing an LNN from rat hippocampal neurons have been described in previous papers [4,5]. The

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method is a modified conventional Banker's method [8]. Briefly, the hippocampal region of a Wistar rat was dissected from on embryonic day 17 (E17) or E18 and neurons were dissociated with 0.175% trypsin (Invitrogen-Gibco, U.S.A.) in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS-minus, Nissui) supplemented with 10 mM glucose at 37℃ for 10 min. Neurons were then seeded in the cloning ling put on the center of a culture dish with 64 planar microelectrodes on the bottom (MED probe, Alpha MED Scientific, Japan) [9]. We used MED probes with 455 µm of inter-electrode distance. The MED probe was precoated with 0.02% polyethylene-imine overnight. The density of the seeded cells was 7800 cells/mm^2. The high density of cells contributes to the survival of the neurons and neurons in the culture can be maintained for more than 100 days. The culture medium was a mixture of Dulbecco's modified minimum essential medium (Invitrogen-Gibco, U.S.A.) and Ham's F12 (Invitrogen-Gibco, U.S.A.), supplemented with 5% horse serum and 5% fetal bovine serum. Half of the culture medium was exchanged with fresh medium every second day. All the procedure of animal experiments was conducted according to "Kwansei Gakuin University animal experiment administrative rules".

B. Electrophysiological recording

The extracellular potentials were recorded through 64 electrodes simultaneously using the integrated MED64 system (Alpha MED Scientific, Japan) at a sampling rate of 10 kHz. Spontaneous activity and action potentials evoked by current stimulation are recorded by the system. The spikes of action potentials were detected automatically using an amplitude threshold-based algorithm for detection on Brain Interface, which we developed program. The threshold was set to be 3-4 fold the level of baseline noise within each 50 ms time windows. Extra large spikes of stimulation artifacts were omitted. A spike-sorting procedure was not performed in Vitroid experiments, because only a small number of neurons were sensed by a single electrode [10], and the spike-sorting procedure requires a great deal of computational cost, preventing real-time processing in the control of a robot body. Electrical stimulation was performed by internal isolators of MED64 system. Stimulation control was also conducted by Brain Interface, according to the stimulation command transmitted by Client program.

C. Vitroid Neuro-robot System

We performed closed-loop interaction between LNN and outer world, interfaced by a neuro-robot hybrid system. The integration of a moving robot and a living neuronal network was firstly proposed by Potter's group as Hybrot [6]. Different from Hybrot, our neuro-robot independently performs a current stimulation to LNN and a detection of the activity of LNN. The detection of action potentials is routinely performed every 50 ms time window, independent to the electrical inputs to LNN. We call this concept of building up the system Vitroid (Fig.2).

LNN was given an artificial body by connecting a robot. A Khepera II robot (K-Team, Swiss) , e-puck(AAI Japan, Japan) or robot consisted by Mind Storm NXT kit (LEGO, U.S.A.) were used for a body of the neuro-robot Vitroid. C# and LabVIEW (National instruments, U.S.A.) were used for the programming language. The program "Brain Interface" performed control of stimulation and event detection. These programs communicated each other by Data socket protocol (National instruments, U.S.A.) [11]. Electrical stimulation and measurement for activity of LNN were performed by multisite recording system for extracellular potentials (MED64 system, Alpha MED Scientific, Japan). Values of IR sensors on the robot and the spatiotemporal pattern of the LNN activity were processed by the program "Client". Client has input interpreter and output interpreter to translate the value of sensors to LNN or to translate the response of LNN to commands for actuator speed. Various algorithms can be selected for the output interpreter, and we have developed Vitroid with simplified fuzzy reasoning, an ANN with Hebbian tuning links and a weighted averaging of selected electrode activity, for the out put interpreters.

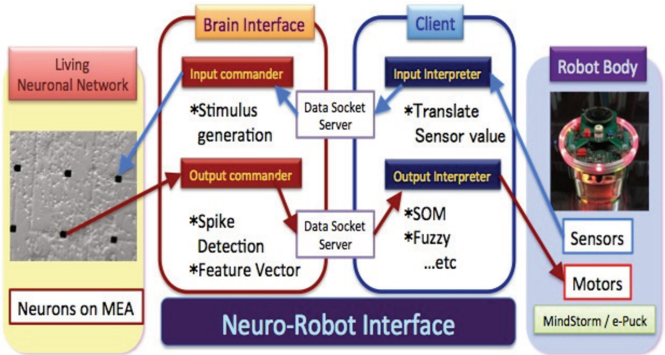

Figure 2. A schematic diagram of neuro-robot system "Vitroid".

III. RESULTS AND DISCUSSION

A. Vitroid with Simplified Fuzzy Reasoning

We previously reported the Vitroid with simplified fuzzy reasoning. We also call this type of Vitroid as "Biomodeling System". Briefly, the output interpreter uses two sets of 256 fuzzy rules for 8 inputs. The two sets of fuzzy rules share a common set of 256 antecedent clauses (if-part) and two distinct sets of 256 consequent clauses. The output interpreter receives 8 inputs from 8 electrodes in LNN. Each input is the number of detected action potentials within 50 ms time window. Each input of the fuzzy reasoning unit has two fuzzy labels, high-frequency and low-frequency. This large number (256) of rules is used in order to describe all the probable patterns for 8 inputs. The maximum number of the input action potential in all electrodes defines the maximum of the horizontal axis of a membership function. The maximum of the membership function assigned to the high-frequency label corresponds to three fourths of the points of maximum frequency, and the maximum of the membership function assigned to a low-frequency label corresponds to one fourth of the points of maximum frequency. These antecedent clauses are used as pattern templates for 8 inputs from LNN. Inputted patterns are compared to these templates, and the compatibility degrees are calculated according to the similarities between the inputted pattern and each template. A motor speed value is then decided as a weighted average of the value of consequent clause (then-part) of each fuzzy rule. To determine the quantitative relationships between the inputted patterns and the speeds of the actuators, we adjusted the consequent clauses of each fuzzy rule by teacher learning. Vitroid succeeded in navigating a course between two parallel walls without any collision with a wall. In the later part of the running, it seemed that collision avoidance delayed gradually (Figure 3a). The variance in number of electrical spikes evoked by inputs tends to decrease during robot movement, though the standard deviation in the number of electrical spikes evoked by spontaneous activity obviously increases. It suggests that the modification of network dynamics is induced by robot behavior, especially in response to the inputs (Figure 3b).

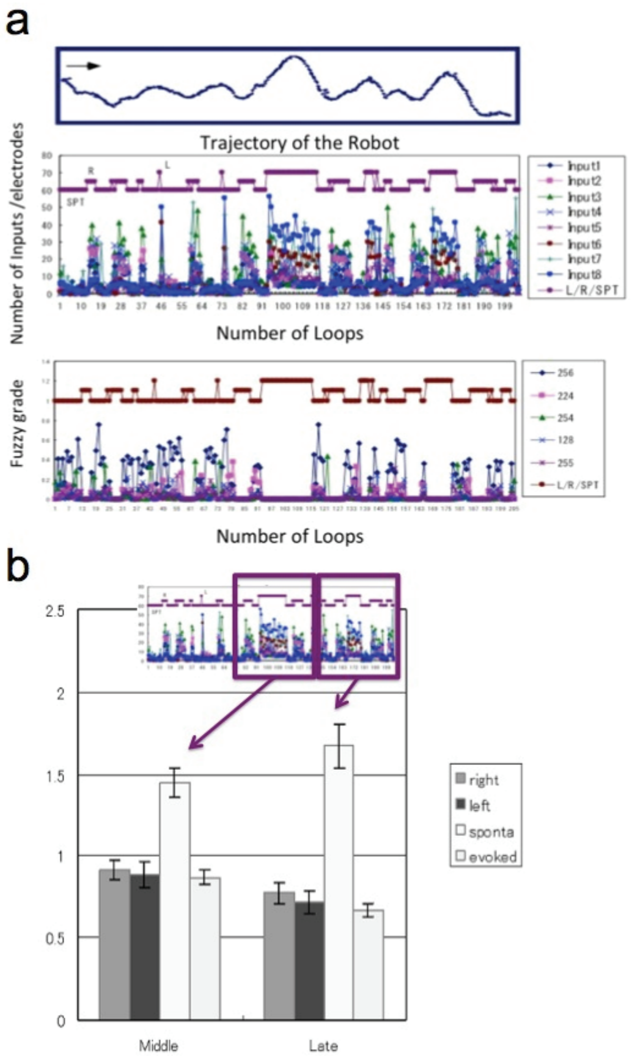

Figure 3. a: An example of the trajectory, the classified inputs (L, R,SPT) and number of inputs corresponding to the activities in eight electrodes, and the Fuzzy grade (compatibility degrees) of the top five rules during an experiment with 1 mM Mg^{2+} recording solution. b: An example of the variance in number of electrical spikes evoked by inputs at middle and late stage of the experiment.

B. *Vitroid with weighted averaging of selected electrode activity*

As an example of the algorithm for output interpreter, we firstly attempted a pattern recognition method with simplified fuzzy reasoning. The interpreter is successful to control a robot body; however, there is a probability that this interpreter is too flexible to induce the modification of network dynamics in LNN. In other words, the output interpreter with fuzzy reasoning can adequately conduct a robot in response to even unsuitable output patterns of the neuronal network. In this case, neurons often receive unsuitable feedback signals as a result of that. So we next investigated the network changes by a simpler method for interfacing neurons and outer world. Weighted averaging of the spike frequency for selected electrodes is one of such simple methods. Firstly, we selected each 3 electrodes which had plenty activity in response to the electrical input, corresponding to obstacles in L side and R side of the robot body. Then suitable weights for 6 values of spike numbers were set for collision avoidance. In the case that response patterns evoked by inputs for L and R obstacles were much discrete, simple weighted averaging of the values of selected electrodes is also effective for simple task, such as a collision avoidance (Figure 4).

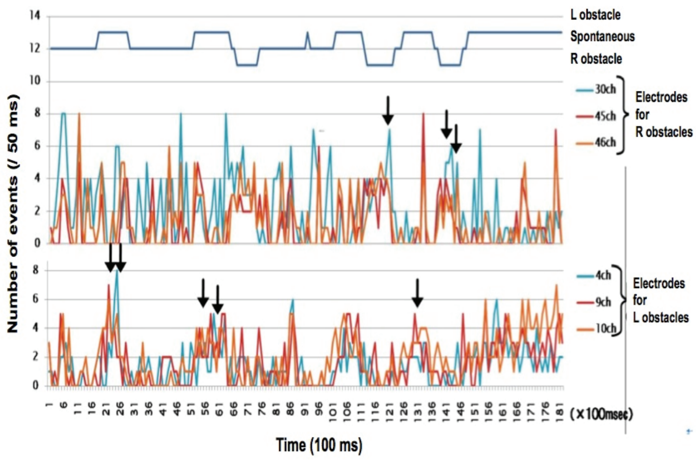

Figure 4. An example of the classified inputs (L, R,SPT) and number of inputs corresponding to the activities in each 3 electrodes for L and R obstacles. Arrows indicated suitable responses for collision avoidance.

We expect that robot behavior generated by a simple weighted averaging causes drastic change in the network dynamics of LNN. However, the result is ambiguous. Though the standard deviation of the number of events in evoked activity show the tendency to decrease during robot running similar with the case of fuzzy reasoning, the standard deviation of the number of events in spontaneous activity also decreased (Figure 5). Spontaneous activity is autonomous and we hardly control. In the experiments using a simple weighted averaging, spontaneous activity occasionally tended to be active and there were a lot of spontaneous activity comparing to the experiments with fuzzy reasoning. The common tendency was decrease of the standard deviation of number of events evoked by inputs during a robot running. It suggests that repetitive electrical stimuli induce synaptic plasticity in LNN and functional connections between neurons enhanced, which prevents synaptic inputs from not evoking an action potential.

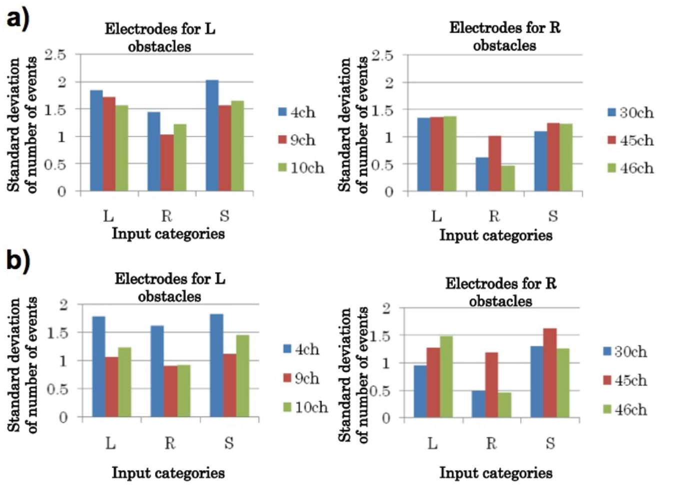

Figure 5. An example of the changes in standard deviations of the number of electrical events. a: Standard deviations of the number of events during the first running of a robot with 60 X 120 cm test course. Left panel indicates the parameters of electrodes for L obstacles and right panel indicates for R obstacles. b: Standard deviations of the number of events during the 65th running of a robot. Descriptions are same with a.

IV. Conclusion

To realize an embodiment of cultured neuronal circuit, we provided cultured neurons with a body of a miniature-moving robot. We call our neuro-robot system as ”Vitroid", meaning a system as a kind of a “test tube” for cognitive agent made by living component. We have developed Vitroid with simplified fuzzy reasoning, and a weighted averaging of selected electrode activity. Both algorithms for linking induced the decrease of standard deviation of the number of electrical events induced by inputs from sensor on the robot, suggesting that the modification of network dynamics is induced by robot behavior, which is likely in animal brain.

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