

A robust pattern of neuronal response to outer phenomena in "Vitroid", the hybrid neuro-robot

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Abstract—Rat hippocampal neurons reorganized a complex networks on microelectrodes array dish. The living neuronal network can distinguish patterns of action potentials evoked by different inputs, suggesting that a cultured neuronal network can represent particular states as symbols. A neuro-robot-hybrid system with living neuronal network and miniature moving robot was developed. We use a Khepera II robot for interfacing with a living neuronal network and the outer world and succeeded in performing collision avoidance behavior with premised control rule sets. Using self-tuning fuzzy reasoning, we associated a distinct spatial pattern of electrical activity with a particular phenomenon in the outside of the culture dish. The particular relationship between network activity and outer phenomenon was performed by control rules of electrical stimulation to the neuronal network, responding to outer phenomenon, while a spatio-temporal patterns of neuronal activity were linked to output devices by premised control rules. We succeeded in performing collision avoidance, and found that fluctuation of neuronal responses evoked by sensor output of robot body was controlled by the interaction between neurons via synapses.

I. INTRODUCTION

Biological intelligence has characteristic functions which artificial intelligence hardly perform yet. Symbol grounding problem, frame problem are often cited as examples of technological difficulties of artificial intelligence reliable in real world. In addition, autonomous generation of algorithms is also one of the features of biological intelligence. Against these oft-expressed problems, "embodied cognitive science" offers some good solutions [1]. In the concept of embodied cognitive science or robotics, some a priori rules for behaviors are embedded in the relationships of sensors and actuators. Circuits are hierarchically connected each other by plastic (in the meaning of availability of adjusting) links. This simple architecture, such as subsumption architecture, often perform amazing adaptability and intellectual behavior [2]. 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 experimental science, but we think that we can construct such system with phenomenal consciousness or qualia by integration of embodied cognitive robotics and self-organizing network components. A quick way to provide such self-organizing network is to use living neuronal network (LNN) reorganized in vitro (fig.1). We can prepare dissociated

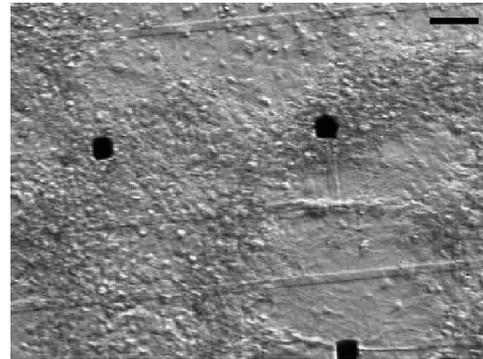


Fig. 1. Example of cultured living neuronal network (E18DIV22). The black bar indicates 100 μ m.

neurons from rat hippocampus and cultivate neurons on culture dish [4]. Cultured neurons elongated neurites and formed a complex network even in the artificial condition [5]. Using culture dish with planar microelectrodes, we observed spontaneous action potentials without any external current inputs, suggesting that interaction between neurons was fully active in the culture dish [6], [7]. The cultured living neuronal network had internal states represented by spatio-temporal pattern of action potentials. In addition, a certain pattern of action potentials were evoked by a current input, corresponding to input from outer world (fig.2). It seems that such an input only "recall" a particular internal state to the network. The relationship between an object (represented by current inputs from sensors) in outer world and an internal state is not strict but loose. The relationship of a particular object and an internal state of neuronal network linked to the object is defined only by reproducibility of such relationship in experience of the cognitive system. This framework is same as definition of "qualia" in "Mind and the world order" by Lewis [8].

II. METHODS

A. Preparation of LNN

Primary cultures of rat hippocampal neurons were used as LNN. The hippocampal region was dissected from Wistar rats on embryonic day 17 (E17) or E18 and neurons were dissociated. Procedure of primary culture of rat hippocampal neu-

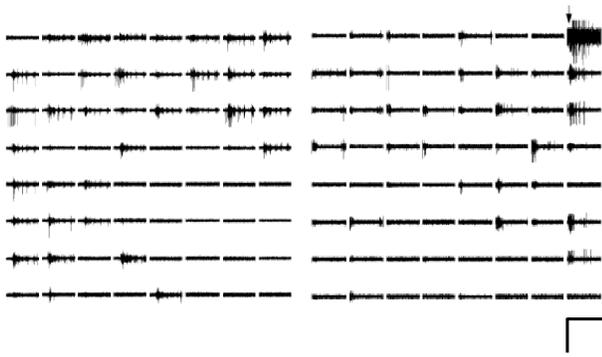


Fig. 2. Examples of spontaneous action potentials (left) and evoked action potentials (right) recorded by 64 electrodes. Scale bar indicates $1 \text{ s} \times 40 \mu\text{V}$.

rons was conventional one and was previously described [4]. Briefly, the rat hippocampal neurons were dissociated by 0.175% trypsin (Invitrogen-Gibco, U.S.A.) in Ca^{2+} - and Mg^{2+} -phosphate-buffered saline (PBS-minus, Nissui) supplemented with 10 mM glucose at 37°C for 10 min. Then neurons were plated on a MED probe (Alpha MED Science, Japan), which is a culture dish with 64 planar microelectrodes on the bottom [9]. The MED probe was precoated with 0.02% poly ethylene-imine overnight. Neurons were seeded in the cloning ring put on the center of a MED probe. Density of seeded cell was $7800 \text{ cells}/\text{mm}^2$. The culture medium was based on the 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 to fresh one every two days. The neurons were cultured for 60 days at 37°C in 5% CO_2 /95% air at saturating humidity. The conduct of all experimental procedures was governed by the Guidelines for the Care and Use of Laboratory Animals of the AIST.

B. Measurement of multiple site extracellular potentials by the electrode array dish

Extracellular action potentials were recorded in the normal culture medium at 20-60 days in vitro. The extracellular potentials were gathered through 64 electrodes simultaneously with the integrated MED64 system (Alpha MED Science, Japan) at a sampling rate of 10 kHz. All experiments were carried out at room temperature ($20\text{-}25^\circ\text{C}$). The spikes of action potentials were detected automatically by amplitude threshold-based algorithm of the detection. The threshold was determined to be 3 times of baseline noise during the 50 msec time window. Extra large spikes of stimulation artifact were omitted. Spike-sorting procedure was not performed in this study, because the small number of neurons were sensed by a single electrode, and spike-sorting procedure requires of much computational cost, preventing the in-time processing to control robot.

C. Design of Vitroid

We performed closed-loop interaction between LNN and outer world, interfaced by a neuro-robot hybrid system. The idea of integration of a moving robot and a living neuronal network was firstly proposed by Potter's group as Hybrot [10], [11], [12]. In their recent papers, robot or simulated robot were controlled according to action potentials evoked within 100 ms after proving electrical stimulus. These "probe stimulus" were applied to the neural network every 5 seconds. In addition they applied random stimuli mimicked constantly derived sensory inputs of animals. They used well-designed stimulation protocols and paid attention to make the neuronal network be stable. Interestingly, they also succeeded in goal-directed learning. Their design of closed-loop interaction look smart for controlling of LNN [13].

On the other hand, we do not stimulate the neuronal network continuously but stimulate only when actual sensors are activated. Our neuro-robot independently treated a current stimulation to LNN and a detection of action potentials. The detection of action potentials was routinely performed every 50 ms time window, independent to the inputs to LNN. This means that the system does not discriminate spontaneous activity and activity evoked by sensor inputs. Indeed, it is difficult to find "pure spontaneous" activities in our brain, because the stream of numerous sensory inputs flow into an animal brain. But neurons cannot distinguish between signals from sensor and signals come from spontaneous activity. These indistinctive action potentials represent internal states of LNN. Input from outer world only recall one of particular state of internal state of LNN. We think a response of neuronal network is not tightly coupled to an input from outer world. The relationship between a signal corresponding to an object in outer world and an internal state is loose and fluctuated. We think these fluctuations are not noise but reflect on a certain type of information processing in LNN. In our paradigm, we do not perform conscious control of the living neuronal network. Instead of that, we adapt interface to LNN. The direction of self-tuning process of LNN is not often suitable for reasonable behavior, so we have to design the interface to match the reasonable behavior and direction of self-tuning process of LNN. We call neuro-robot system with such direct-coupling type of interface as "Vitroid". The system is a sort of "test tube" for cognitive agent made by living component. LNN is main processing unit of Vitroid and all decision-making of the system is performed by this LNN, including learning. Vitroid possesses at least 2 interpreters. An "output interpreter" translates detected activity patterns of LNN into behaviours of robot. An "input interpreter" translates outside phenomena into electrical stimulation to LNN. We embedded rules for reasonable reaction against outside object into these interpreters, just like couplings between actuator and sensor pairs in robot designed by embodied cognitive science. There are many candidate of algorithm for transforming the spatio-temporal pattern of action potentials to control value. Currently

self-tuning fuzzy reasoning is used for output interpreter in this study [14]. The output interpreter uses parallel 2 sets of 256 fuzzy rules (fig.3). The 2 set of fuzzy rules have a common

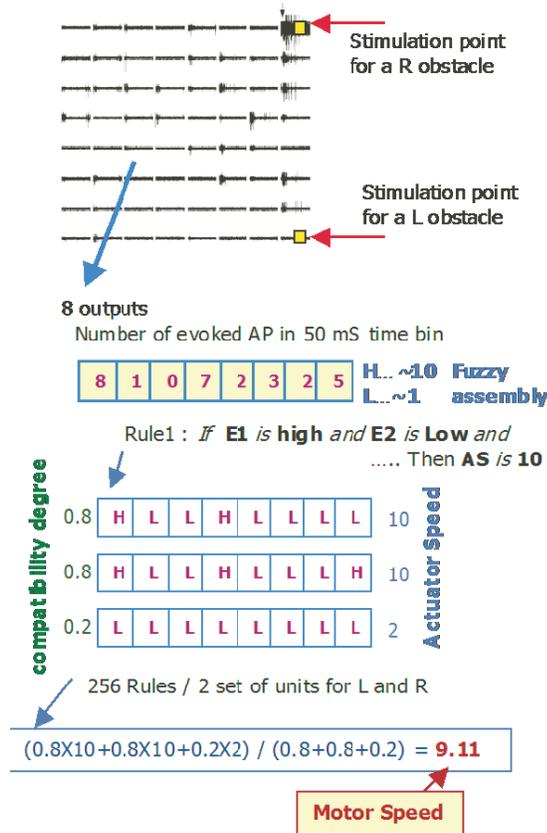


Fig. 3. Implementation of simplified fuzzy reasoning in output interpreter.

set of 256 antecedent clauses (if-part) and 2 distinct set of 256 consequent clauses. The output interpreter receives eight signals from LNN. Each signal is the number of detected action potentials per 50 ms time windows from 8 electrodes. Each input of fuzzy reasoning unit has two types of fuzzy labels, high-frequency and low-frequency. 256 fuzzy rules are constituted by 8 inputs with high-freq. and low-freq. fuzzy labels. 256 fuzzy rules is over-spec for only two states recognition, but this large number of rules is in order to describe all classified patterns of eight inputs. That is required rather for analysis of neuronal activity, not for control of actuators. The maximum frequency of the action potential in all electrodes is made the maximum of the horizontal axis of a membership function. The maximum of membership function assigned to the high-frequency label is at three fourths of the points of maximum frequency, and the maximum of the membership function assigned to a low-frequency label was at one fourth of the points of maximum frequency. For simplification, each membership function for all 8 ch inputs is the same function currently. The common 256 antecedent clauses are used as pattern templates in the output interpreter. Inputted pattern is compared to these templates, and compatibility degrees are calculated according to similarity between inputted pattern and

each template. Compatibility degree of each rule (template) is large when spatial pattern of evoked action potentials is similar to the template. Then a value of motor speed is decided as weighted average of value of consequent clause (then-part) of each fuzzy rule, by following equation;

$$h_n = \prod_{k=1}^8 \mu_{A_k}(x_k) \quad (1)$$

$$z_o = \frac{\sum_{n=1}^{256} z_n \cdot h_n}{\sum_{n=1}^{256} h_n} \quad (2)$$

where z_o represents output value of fuzzy reasoning, z_n represents a value of the consequent clause of each rule, h_n represents a compatibility degree of each rule, μ_{A_k} represents a fuzzy number of each input in each rule, and x_k represents each inputted value to fuzzy reasoning. Fuzzy reasoning is not necessarily best much for the system because requirement is a kind of a pattern recognition. To use fuzzy reasoning is reasonable only when there is a quantitative relationship between an inputted value and an output control value. For example, If the system should be designed to make an output value "speed of actuator" strongly decrease by an inputted value "a curve is very acute", fuzzy reasoning works effectively. However, inputted value, a pattern of evoked action potentials has no relationship to speed of an actuator in Vitroid. To make such quantitative relationships between feature of inputted pterns and speeds of actuators, we adjust consequent clause of each fuzzy rule by teacher learning. The tuning of consequent clause by teacher learning is performed by minimization of differences between teacher signal and output value of each fuzzy reasoning. Learning unit generates 3 categories of stimulation signals (L or R or No stimulation) and optimal speeds of actuators as a teacher signal. Then electrical stimulations are applied to a LNN, and corresponding responses are gathered and inputted in a fuzzy reasoning unit. Out put of the fuzzy reasoning is assessed and the value of a consequent of the each rule is adjusted. This consequent tuning is performed by;

$$z_n = z_n + \tau \cdot h_n \cdot (z_t - z_o) \quad (3)$$

for all learning trials $i = 1, 2, \dots, n$. Where z_n represents a value of the consequent clause, and h_n represents a compatibility of degree each rule. τ represents a learning coefficient, which desides quantity of the adjustment, z_t represents a teacher signal (target value) for output of fuzzy reasoning, and z_o represents output value of fuzzy reasoning. As a result of that, the values of consequent clauses are adjusted mainly by similarity between inputted pattern and target pattern (Target pattern means the pattern repeatedly evoked by a particular input, and most general and most general one). Another dominant factor of adjustment is independency of the inputted pattern against stimulation category (L or R).

On the other hand, simple summation of IR sensor value is used for input interpreter. The value of 3 IR sensors at a

left side and 3 IR sensors at a right side of the robot body are summed respectively. Then the summation of the value is compared. If a value of summation of left side sensors exceeds a value of summation of right side sensors plus threshold, input interpreter stimulate a LNN via an electrode assigned to "left" in advance, vice versa. The parameters of these interpreters are fixed, meaning that the artificial part of framework of vitroid is not plastic. Even though LNN and interpreters initially adapted each other quite well, plastic feature of LNN modified the matching between LNN and artificial framework. As a result of that, there is a possibility that information processing in LNN changes to inadequate for reasonable behavior. We focused on the change of dynamics of LNN, expected to suit for reasonable behavior of Vitroid.

D. System integration of Vitroid

We integrated a robot body, control computers, electrophysiological components and a network of rat hippocampal neurons(fig.4). We use a Khepera II robot (K-Team) or Robot constructed by LEGO mindstorm NXT kit for a body of Vitroid. We used multisite recording system for extracellular potentials [15], [16], [17] (MED64 system, Alpha MED Science) as electrophysiological components, which include 64 signal amplifiers, 2ch of integrated stimulators and integrated A/D-D/A converters (multifunctional data acquisition circuits). Computers are employed for controlling electrophysiological components via multifunctional data acquisition circuits, and for controlling a robot via RS232C interface. Fuzzy reason-

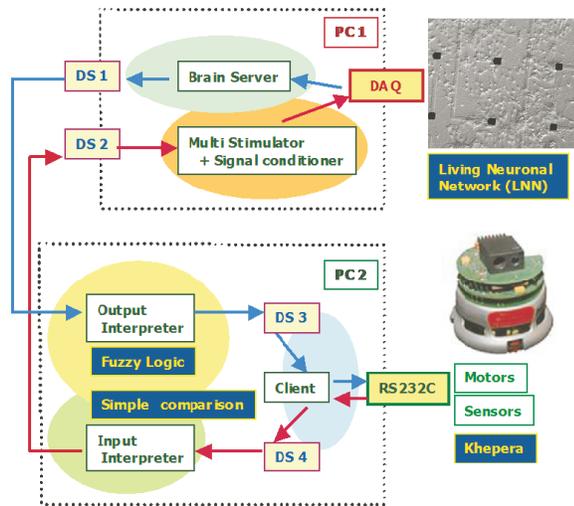


Fig. 4. System integration of Vitroid. Vitroid is constructed by 5 programs running on 2 computers, a LNN and a robot body. An information flow form a robot to LNN (afferent pathway) and an information flow form a LNN to a robot (distal pathway) constitute closed-loop.

ing programs were implemented in an output and an input interpreter, respectively. The "Client" program controled a robot body and read the sensor values of a robot body. The "multi Stimulator" program stimulates the neuronal network according to stimulation pattern command generated by an input interpreter. Programs exchange processed data information

mediated by a datasocket transfer protocol (DSTP, National Instruments).

E. Test run of Vitroid

After tuning of parameters of interpreters, we make Vitroid run experiments in collision avoidance. Vitroid was put on between 2 walls arranged at parallel, then we turned on the switch of connecting the robot body and a LNN. The experiment was stopped when Vitroid failed to avoid collision to the wall, or Vitroid reached to the end of the test course. Experiments were performed under the condition that the concentrations of $MgCl_2$ in the extracellular recording solution of a LNN were 1 mM and 5 mM. Culture medium was DMEM/F12 medium base and DMEM/F12 medium includes 0.7 mM Mg^{2+} . The culture medium included about 1mM Mg^{2+} , though accurate concentration of Mg^{2+} is unknown because of addition of serums. So, 1 mM $MgCl_2$ concentration is normal condition for extracellular solution of a LNN, while 5 mM of $MgCl_2$ concentration is a comparatively high concentration. The activities of divalent cation channels, such as Ca^{2+} channel and NMDA-type glutamate receptors, decrease under such high Mg^{2+} concentration [18].

As a result of that, frequency of spontaneous activity decreases, meaning background activity irrelevant to inputs form outer world decreases. We compared responses of a LNN to input from outer world in 1 mM and in 5 mM Mg^{2+} recording solution.

III. RESULTS AND DISCUSSIONS

A. Teacher Learning of output interpreter

Before test-run, an output interpreter of Vitroid should be trained. 150 trials of stimuli were applied to a LNN for learning of consequent clauses of fuzzy rules. Initial value of consequent clause of each rule equally set at 5. Teacher signals corresponding to an obstacle in left side were 10 for a left actuator and 1 for a right actuator. Teacher signals corresponding to obstacles in right side were 1 for a left actuator and 10 for a right actuator. Because of this symmetrical setting of teacher signals, distributions of actuator speed in consequent clauses were in contrast with the speed of counter actuator(fig.5). Learning was performed in 5mM Mg^{2+} recording solution in order to reduce spontaneous activity, which obfuscates the learning. Adjusted values of consequent clauses did not completely converge during 150 trials of learning, but we gave priority to avoid damage of neurons by repeated current stimuli.

B. Collision avoidance of Vitroid

Vitroid succeeded in performing the zigzag run of between two walls arranged at parallel, without collision with a wall. In later half of experiments, it seemed that collision avoidance delayed gradually (fig.6). The several reasons can be considered about that; first is delay of communication between each unit of a vitroid system. Second is wrong modifications of neuronal responses to inputs (L or R). Correspondences between labels for inputs to IR sensors and responses of a

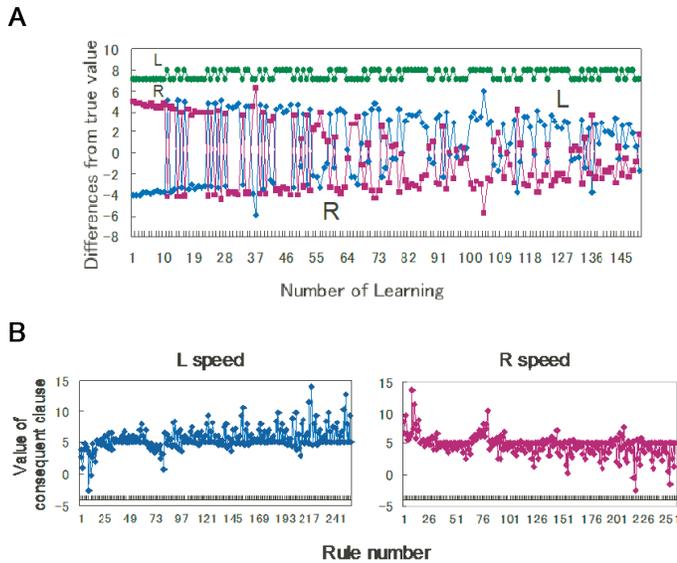


Fig. 5. An example of teacher learning of fuzzy reasoning of output interpreter. A. teacher signal and value of consequent clause for L and R actuators of each trial during teacher learning. B. Distributions of 256 values of consequent clauses for L and R actuators.

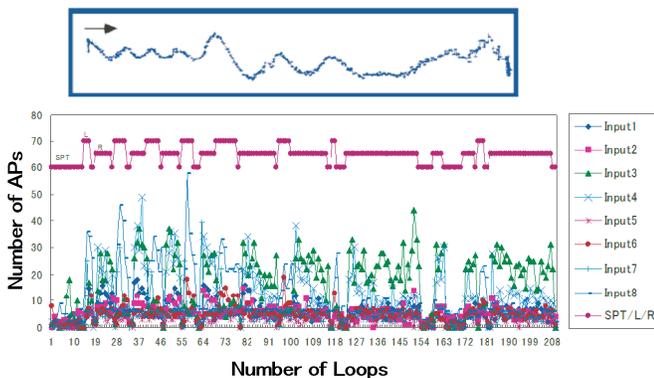


Fig. 6. An example of collision avoidance of vitroid under 5mM Mg^{2+} condition. Upper panel indicates a trajectory of robot body during an experiment. Lower panel indicates number of action potentials in 50 mS time windows. Upper trace indicates labels determined by IR sensors of a robot body. Lower traces are responses of 8 neurons recorded by 8 microelectrodes.

LNN were changed to be rather stable in later half. So, it seems that delays of communications were influenced on the collision avoidance. That point should be improved. The responses of neurons to inputs from outer world were relatively stable. The result suggests that inputs linked to "L-side obstacles" or "R-side obstacles" evoked reproducible pattern of electrical activity, even though spontaneous autonomous activity varied during experiments. This reproducibility, however, was less than perfect, because the spatiotemporal pattern of the network activity was determined not only by input stimulation but also spontaneous internal states of the network. This spontaneous activity reflected the degree of fluctuation of the internal state of a LNN. When concentration of Mg^{2+} in recording solution was reduced to 1mM, frequency of the spontaneous

activity increased drastically, suggesting that fluctuation of the internal state also increased (fig.7). Our expectation of

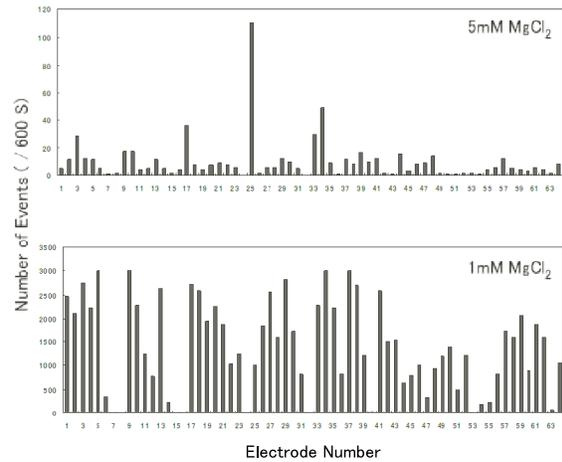


Fig. 7. Drastic elevation of spontaneous activities by reduced Mg^{2+} condition. A LNN is same one in both condition, E18DIV29.

stability of responses of neurons to sensor inputs was that the stability would be abolished and responding pattern of neurons would be confused. However, the responses of neurons to sensor inputs were rather more stable than in 5mM Mg^{2+} (fig.8). This suggests that the spatio-temporal pattern of action

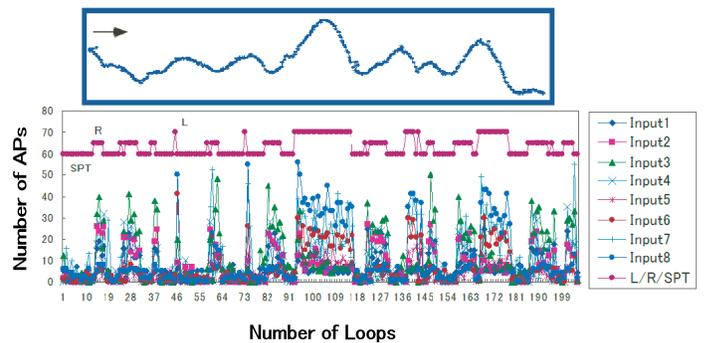


Fig. 8. An example of collision avoidance of vitroid under 1mM Mg^{2+} condition. format and symbols are same as in fig.ref5.

potentials evoked by sensor inputs was identical and stable equally in 1mM and 5mM Mg^{2+} , even though fluctuation of the internal state increased in 1mM Mg^{2+} . Even in a highly fluctuating state, LNN seemed to have mechanisms for adjusting stabilization of evoked response.

This feature of LNN is thought to be one of the fundamental function of animal brain for flexible adaptation to outer world. Now we are analyzing the mechanism, using Vitroid, our interaction system between neurons and outer world.

IV. CONCLUSION

The living neuronal network was able to distinguish patterns of action potentials evoked by different inputs. Using self-tuning fuzzy reasoning, we associated a distinct spatial pattern of electrical activity with a particular phenomenon in the

outside of the culture dish and Vitroid, our robot system with interpreter units and a living neuronal network, succeeded in performing collision avoidance. In addition, the spatio-temporal pattern of action potentials evoked by sensor inputs was identical and stable even under the highly fluctuating internal state. The system is a good testing platform for clarifying interaction between living neurons and the outer world.

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REFERENCES

- [1] R. Pfeifer and C. Scheier: Understanding Intelligence *The MIT Press*, 1999.
- [2] R. Brooks: A robust layered control system for a mobile robot. *IEEE Journal of Robotics and Automation*, **Vol.2** 1, pp.14-23, 1986.
- [3] C.I. Lewis: Mind and the world order *C. Scribner's Sons, New York* 1929.
- [4] G.A. Banker and W.M. Cowan: Rat hippocampal neurons in dispersed cell culture. *Brain Res*, **126**, pp.397-342, 1977.
- [5] S. N. Kudoh, A. Matsuo, K. Kiyosue, M. Kasai and T. Taguchi: Long-lasting enhancement of synaptic activity in dissociated cerebral neurons induced by brief exposure to Mg^{2+} -free conditions. *Neurosci Res*, **28**, 4, pp.337-344, 1997.
- [6] G. W. Gross, A. N. Williams and J. H. Lucas: Recording of spontaneous activity with photoetched microelectrode surfaces from mouse spinal neurons in culture. *J Neurosci Methods*, **5**, 1-2, pp.13-22, 1982.
- [7] A. Wagenaar Daniel, J. Pine and M. Potter Steve: An extremely rich repertoire of bursting patterns during the development of cortical cultures. *BMC Neurosci*, **7**:11, pp. doi10.1186/1471-2202, 2006.
- [8] G. Banker and K. Goslin: Culturing Nerve Cells (Cellular and Molecular Neuroscience Series) *MIT press*, 1998.
- [9] H. Oka, K. Shimonio, R. Ogawa, H. Sugihara and M. Taketani: A new planar multielectrode array for extracellular recording: application to hippocampal acute slice. *J Neurosci Methods*, **93**, 1, pp.61-67, 1999.
- [10] J. D. Bakkum, C. A. Shkolnik, G. Ben-Ary, P. Gamblen, P. T. B. DeMarse, and M. S. Potter: Removing some 'A' from AI: Embodied Cultured Networks. *Embodied Artificial Intelligence*. Iida, F., Pfeifer, R., Steels, L. and Kuniyoshi, Y. *New York, Springer. New York, Springer*, **3139**, pp. 130-145, 2004.
- [11] J. D. Bakkum, M. P. Gamblen, G. Ben-Ary, C. Z. Chao and M. S. Potter : MEART: The Semi-living Artist. *Frontiers in NeuroRobotics*, **1:5**, pp.1-10,doi.10.3389/neuro.12.00, 2007.
- [12] B. T. DeMarse, A. D. Wagenaar, P. A. W. Blau, and M. S. Potter: The Neurally Controlled Animat: Biological Brains Acting with Simulated Bodies. *Autonomous Robots*, **11**, pp.305-310, 2001.
- [13] Z. C. Chao, D. J. Bakkum and S. M. Potter: Shaping embodied neural networks for adaptive goal-directed behavior. *PLoS Comput Biol*, **4**, 3, e1000042, 2008.
- [14] E. H. Mamdami: Application of fuzzy algorithms for the control of a simple dynamic plant. *Proc IEEE*, pp.121-158, 1974.
- [15] J. Pine: Recording action potentials from cultured neurons with extracellular microcircuit electrodes. *J Neurosci Methods*, **2**, 1, pp.19-31, 1980.
- [16] G. W. Gross, E. Rieske, G. W. Kreutzberg A. Meyer : A new fixed-array multimicroelectrode system designed for longterm monitoring of extracellular single unit neuronal activity in vitro. *Neurosci Lett*, **6** , pp.101-105, 1977.
- [17] Y. Jimbo, H. P. Robinson and A. Kawana: Simultaneous measurement of intracellular calcium and electrical activity from patterned neural networks in culture. *IEEE Trans Biomed Eng*, **40**, 8, pp.804-810, 1993.
- [18] H. Kamioka, E. Maeda, Y. Jimbo, H. P. Robinson and A. Kawana: Spontaneous periodic synchronized bursting during formation of mature patterns of connections in cortical cultures. *Neurosci Lett*, **206**, 2-3, pp.109-112, 1996.