WC3-5

Vitroid - a robot with link between living neuronal network in vitro and robot body

Suguru N. Kudoh*, Minori Tokuda3, Ai Kiyohara[†], Chie Hosokawa*, Takahisa Taguchi* and Isao Hayashi[‡] *Cell engineering R.I.AIST,

1-8-31 Midorioka, Ikeda, Osaka 563-8577 Email: s.n.kudoh@aist.go.jp

[†]Cell engineering R.I.AIST / Osaka univ.

[‡] Fac. infomatics, Kansai univ., 2-1-1 Reizenji-cho, Takatsuki, Osaka 569-1095

Abstract—Rat hippocampal neurons organized complex networks on a culture dish which has 64 planar microelectrodes and the spontaneous action potentials were frequently observed. The living neuronal network was able to distinguish patterns of action potentials evoked by different inputs, suggesting that a cultured neuronal network can represent and process particular states as symbols. We use a Khepera II robot and a robot made by LEGO mindstorm NXT kit as a robot body for interfacing with a living neuronal network and the outer world. We call the system "Vitroid". Vitroid has the living neurons, robot body, and direct coupling type of controllers to interface neurons with the robot. We succeeded in performing obstacle 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.

I. INTRODUCTION

Information processing in brain system is carried out by electrical interaction between neurons. The spatiotemporal pattern of a neuronal activity is express a certain meaning in a brain [1], [2]. We analysed the spatio-temporal pattern of spontaneous action potentials [3] by multi-site recording system for extracellular potential [4], [6], [5], [3], [7], [8] (Fig. 1).

• •

Fig. 1. Example of cultured living neuronal network (E17D10). The white bar indicates 50 μ m. White arrowheads indicate planar microelectrodes, arranged in an 8 x 8 grid on the culture dish. Example of neuron is indicated (white arrow)

Using the system, we elucidated that the living neuronal network was able to discriminate several patterns of evoked action potentials according to different inputs. The living neuronal network can express several patterns independently. The result suggested that the network of dissociated neurons has fundamental mechanisms for some intelligent information processing. So we are developing the system where living neuronal network connected to moving robot with premised control rules corresponding to genetically provided interface of neuronal network to peripheral system. Such idea, "hybrot" was firstly proposed by Potter's group [9], [11]. Hybrot is controlled depending by new statistic, the Center of neural activity (CA) of 100 ms of responses after each electrical stimulus. Sensory feedback is performed by "Patterned Training Stimulation (PTS)". PTS is repetitive electrical stimulation to induce synaptic plasticity. If the robot or the robot hand is located at illegal position, then PTS is applied into the neuronal network. PTS modify synaptic weight of neurons in the network, and as a result of that, it tunes responses to the proving stimuli [11].



Fig. 2. A direct coupling type of closed-loop interaction.

Such a feed-back system of hybrot consists of a certain type of "closed-loop" interaction. We here proposed other type of "closed-loop" interaction. Information from outer world reflects not only on modification of synaptic strength but also on the internal state of neuronal network. In our system, electrical stimuli are almost directly coupled to input from outer world via interpreter units for translating neuronal signal to behavior and outer phenomena to electrical inputs, and the internal state of the network linked to phenomena in outer world. We omitted proving stimuli for estimation of internal state. Instead of that, we estimate internal state by spontaneous activity and activity evoked by electrical stimuli reflected on inputs from outer world. We call the neuro-robot system "Vitroid", which has in vitro living neuronal networks and such a direct coupling type of closed-loop interaction. In this study, we use a Khepera II robot for the body of the system and succeeded in performing obstacle avoidance behavior with premised control rule sets.



Fig. 3. Schematic diagram of Neuro-Robotic hybrid system. The system consists of 5 independent programs and recording system for multiple-site extracellular potentials, two computers and robot body.

II. SYSTEM INTEGRATION FOR VITROID

We use a Khepera II robot (K-Team) or Robot constructed by LEGO mindstorm NXT kit for interfacing with a living neuronal network and the outer world and LabVIEW (National instruments) for the programming language. Central processing unit of Vitroid is living neuronal network. The system requires at least 2 interpreters; one is an "output interpreter" translating behaviors of robot into electrical activity patterns and another is an "input interpreter" translating outer phenomena into electrical stimulation [13], [12]. Input from the 6 IR sensors of the robot is translated into electrical stimulation pattern by an input interpreter and the stimulation applied into the living neuronal network, evoking a particular activity pattern of action potentials corresponding to the sensor value. The system then determines speeds of two actuators by output interpreter according to the spatial pattern of electrical activity of neurons.

There are the candidates of such a translation algorithm, and we adopted self-tuning fuzzy reasoning in this study. The particular relationships between network activity and outer phenomenon were formed by control rule set of electrical stimulation to the neuronal network, responding to outer phenomenon. The system has two fuzzy reasoning units (as input interpreters) for determination of stimulation patterns for two electrodes, and two parallel self-tuning fuzzy reasoning units (as output interpreters) for speed control of light and left actuators.

Two set of self-tuning fuzzy reasoning units of output interpreter receive eight inputs from the living neuronal network.



Fig. 4. Spatiotemporal pattern of evoked and spontaneous activity of neuron linked to speed of actuators on a robot body.

Each input is the number of detected action potentials / 50 ms time windows. These fuzzy controllers consist of 256 fuzzy rules with eight inputs. Each input has two types of fuzzy labels– high-frequency and low-frequency. Too large number of 256 fuzzy rules is in order to describe all classified patterns of action potentials in eight inputs. That is required rather for analysis of neuronal activity, not for control. The maximum frequency of the action potential in all electrodes is made the maximum of the horizontal axis of a membership function. The maximum membership function assigned to the highfrequency 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. Each membership function for all 8 ch inputs is the same function (Fig. 2).



Fig. 5. An experimental setting to perform Collision avoidance.

An output value of speed of actuator is determined by simplified fuzzy reasoning, of which consequent values are defined not as a membership function but as a crisp value. Each value of consequent clause is tuned by supervised learning. The tuning is performed by minimization of differences between teacher signal and output value of conclusion. Learning unit generates stimulation signals to a neuronal network and optimal speeds of actuators as a teacher signal, according to stimulation signal. Then the system applys electrical stimulation to neurons and modifies each value of consequent clause according to teach signal. After a process of learning, interface units is tuned for their brain, an individual living neuronal



Fig. 6. A. An example of trajectory of Vitoroid robot body. B. Label of classified inputs (L,R,SPT) and number of inputs, corresponding activities in 8 ch electrodes. C. Compatibility degrees of top 5 rules during an experiments. The experiment is same as one indicated in B.

network. This learning process make a certain rule to response to outer world, in other words, the process offer "meaning" to Vitroid. The system consists of 5 independent programs and recording system for multiple-site extracellular potentials, and two computers (Fig. 3). The "Brain Server" program records electrical potentials and detects action potentials of neurons from 8 ch of electrodes. FLTD and FLBU programs implement an output and an input interpreter, respectively. The "Client" program controls the robot. The "Input Com" program stimulates the neuronal network according to stimulation pattern command generated by FLBU. Programs exchange processing data information mediated by a datasocket transfer protocol (DSTP, National Instruments) [14]. The system uses 4 datasocket servers without buffering data. So there may be a probability of lost data. It is the second-best policy for avoiding increased time delay between the living neuronal

network and robot.

III. COLLISION AVOIDANCE CONTROLLED BY A LIVING NEURONAL NETWORK

Neurons were once dissociated and cultured on a dish, abolishing any physiological synaptic connections among them. It means that adequate links between a neuronal network and the outer world were corrupted. Interpreters in Vitroid complement such abolished links. When output interpreter detects a particular pattern of network activity, evoked by an electrical stimulation of a particular electrode which is linked to the high value of sensors on either side, the actuator speed on that side is speeded up. Where sensor input is absent, spontaneous electrical activity is generated only by the internal state of the living neuronal network (Fig. 4) [15]. All the rules in interpreters are consisted by learning process of fuzzy-tuning, instead of evolution in nature. The rules also generate spontaneous behavior of Vitroid. Spontaneous activity is classified into particular patterns, and Vitroid moves spontaneously (Fig. 4).

Adequately tuned Vitroid succeeded in performing the zigzag run of between two walls arranged at parallel, without collision with a wall (Fig. 5,6). Inputs linked to "Lside obstacles" or "R-side obstacles" evoked stable pattern of electrical activity, while spontaneous autonomous activity rather fluctuated during experiments. Compatibility degrees discriminated signal by L-side obstacles from R-side obstacles, but the combination pattern of compatibility degrees was much varied. The result means that a certain recognizable pattern is expressed by several different sets of rules. The spatiotemporal pattern of the network activity is determined not only by input stimulation but also spontaneous internal states of the network [15]. This is why the pattern evoked by electrical stimulation is also not constant.

IV. CONCLUSION

We integrated a living neuronal network and a robot body using interpreter. We call that neuro-robot system "Vitroid", which has in vitro living neuronal networks and a direct coupling type of closed-loop interaction.

Vitroid succeeded in performing collision avoidance. The system is a good modeling platform system for clarifying interaction between living neurons and the outer world and assessing the effects of the outer environment on the activity of the living neuronal system.

ACKNOWLEDGMENT

This research is supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan under Grant-in-Aid for Scientific Research 20034060 (priority area "System Cell Engineering by Multi-scale Manipuration"),19200018 and 18500181.

REFERENCES

- D.O Hebb, *The organization of behavior*. (Wiley, New York, 1949):John Wiley, 1949.
- [2] Y.Ikegaya, G.Aaron, R.Cossart, D.Aronov, I.Lampl, D.Ferster, R.Yuste, Synfire chains and cortical songs: temporal modules of cortical activity. *Science.*, Vol. 304, pp.559-564, 2004.
- [3] H.Kamioka, E.Maeda, Y.Jimbo, HP.Robinson, and A.Kawana, Spontaneous periodic synchronized bursting during formation of mature patterns of connections in cortical cultures. *Neurosci Lett*, Vol. 206, pp.109-112, 1996.
- [4] GW.Gross, E.Rieske, GW.Kreutzberg, and A.Meyer, A new fixed-array multimicroelectrode system designed for long-term recording. *Neurosci Lett*, Vol.6, pp.101-105, 1977.
- [5] Jerry Pine, Recording action potentials from cultured neurons with extracellular microcircuit electrodes. J. Neurosci Methods, Vol.2, pp.19-31, 1980.
- [6] 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*, Vol.40, pp.804-810, 1993.
- [7] H.Oka, K.Shimono, R.Ogawa, H.Sugihara, and M.Taketani, A new planar multielectrode array for extracellular recording: application to hippocampal acute slice. *J.Neurosci Methods*, Vol.93, pp.61-7, 1999.
- [8] S.N Kudoh and T.Taguchi, Operation of spatiotemporal patterns stored in living neuronal networks cultured on a microelectrode array. 2003) 8(2):100-107. Journal of Advanced Computational Intelligence and Intelligent Informatics, Vol.8, pp.100-107, 2003.

- [9] T.B.Demarse, D.A.Wagenaar, A.W.Blau, and S.M.Potter, The neurally controlled animat, biological brains acting with simulated bodies. *Autonomous Robots*, Vol.11, pp.305-310, (2001)
- [10] D.J.Bakkum, A.C.Shkolnik, G.Ben-Ary, P.Gamblen, T.B.DeMarse, and S.M.Potter, Removing some 'A' from AI: Embodied Cultured Networks. in *Embodied Artificial Intelligence. F.Iida, R.Pfeifer, L.Steels and Y.Kuniyoshi*New York, Springer. 3139: pp.130-145, 2004.
- [11] MEART: The semi-living artist, D.J. Bakkum, P.M. Gamblen, G.Ben-Ary, Z.C.Chao and S.M.Potter Frontiers in Neurorobotics, Vol.1,A-5, 2007
- [12] Kudoh.SN. Hayashi.I, and Taguchi.T, Synaptic potentiation re-organized functional connections in a cultured neuronal network connected to a moving robot. Proc. of the 5th International Meeting on Substrate-Integrated Micro Electrode Arrays (MEA2006), pp.51-52, 2006.
- [13] S.N.Kudoh, C.Hosokawa, A.Kiyohara, T.Taguchi and I.Hayashi, Biomodeling system - interaction between living neuronal networks and the outer world. *J. Robotics and Mechatronics*, Vol.19(5), pp.592-600, 2007.
- [14] National Instruments Inc., Using DataSocket Technology. in LabVIEW User Manual, pp18-2 - 18-11, 2003.
- [15] P.S. Wolters, W.L.Rutten, G.J.Ramakers, J.Van Pelt, M.A.Corner, Longterm stability and developmental changes in spontaneous network burst firing patterns in dissociated rat cerebral cortex cell cultures on multielectrode arrays. *Neurosci Lett.*, Vol.361, pp.86-9, 2004.