Vitroid – the robot system with an interface between a living neuronal network and outer world

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Abstract: We have developed a neuro-robot-hybrid system using a living neuronal network and a miniature moving robot. The living network of rat hippocampal neurons can distinguish patterns of action potentials evoked by different inputs, suggesting that a cultured neuronal network can represent particular states as symbols. We used a Khepera II robot and a robot made using a LEGO mindstorm NXT kit to interface with a living neuronal network and the outer world. We call the system 'vitroid'. Vitroid has living neurons, a robot body, and direct coupling controllers to interface the neurons with the robot. Vitroid was able to perform obstacle avoidance behaviour with premised control rule sets.

Keywords: MEA; dissociated culture; neuron; Khepera; embodiment; fuzzy reasoning.

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1 Introduction

The intelligence of animals performs critical functions which artificial intelligence is yet unable to match. Such functions include symbol grounding problems or frame problems, which are often cited as examples of technological themes for artificial intelligence. An effective approach to these problems is 'embodied cognitive science' (Brooks et al., 1986; Pfeifer and Scheier, 1999). In embodied cognitive science, or robotics, circuits are hierarchically connected to each other by plastic (in the sense of adjustable or malleable) links. This simple architecture, such as subsumption architecture (Brooks et al., 1986), often exhibits amazing adaptability and intelligent behaviour.

The concept of 'embodiment' includes three basic ideas, as follows:

1 Some a priori rules for instinctive behaviours of an agent (for example, run away when a collision occurs) are 'embedded' in the relationships between the sensors and actuators of the agent. For example, if a value of collision sensor placed at right side of the agent is directly transmitted as a speed to the right actuator, an object at the right side of the agent makes the agent turn to the left. In the case of an animal, these rules are provided by evolution.

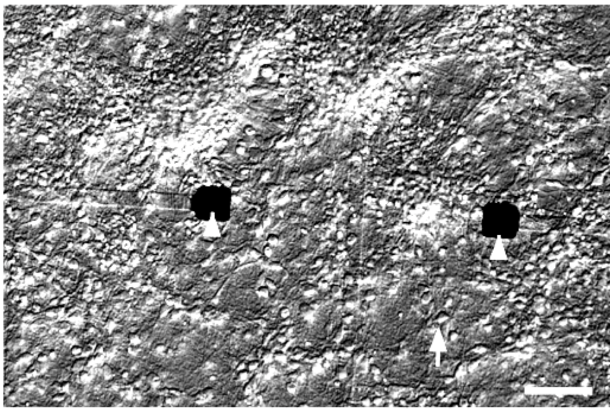
2 The impression of an object in the outer world is divided into features detected by sensors. For example, a 'heavy, blue' object would be automatically divided into a colour feature (from photoreceptor input) and a mass feature (from somatosensory input) by existing sensors.

3 The output of the brain is recursively inputted into the brain as sensory inputs influenced by actions of the body (closed-loop interaction).

The concept of embodiment provides an adequate relationship between the behaviours of the agent and the environment, and enables the emergence of autonomous behaviours. A remaining problem for realisation of creature-like intelligence is how to generate 'phenomenal consciousness' using artificial components. This philosophically challenging problem is difficult to discuss in a field experimental science, but such a system can be constructed with phenomenal consciousness or 'qualia' (Lewis, 1929) through the integration of embodied cognitive robotics and self-organising network components. One way to provide such a self-organising network is to use a cultured living neuronal network (LNN) reorganised in vitro (Figure 1). Dissociated cell culture is a method for maintaining the living cells in artificial environment being out of the living body.

The cultured LNN possesses internal states represented by a spatio-temporal pattern of action potentials (Ikegaya et al., 2004; Wolters et al., 2004; Wagenaar, 2006). In addition, a certain pattern of action potentials is evoked by a current input, corresponding to input from the outer world. It seems that such an input appears to just 'recall' a particular internal state of the network. The relationship between an object (represented by current inputs from sensors) in the outer world and an internal state is not strict but loose (Hebb, 1949). The relationship between a particular object and an internal state of the neuronal network linked to the object is defined only by the reproducibility of such a relationship in the experience of the cognitive system. This framework is the same as the definition of 'qualia' in *Mind and the World Order* by Lewis (1929). We propose that the internal state itself is the main constituent of information processing in the network. We tried to 'link' that internal state of the network with a phenomenon in the outer world. Thus, our aim is to elucidate how the internal state becomes grounded in the real world.

Figure 1 An example of a LNN (E18DIV26)



For this purpose, we performed a closed-loop interaction between the LNN and the outer world, using a neuro-robot hybrid system. The idea of integration of a moving robot and a LNN providing for a closed-loop interaction between a neuronal network and the outside world was first proposed by Potter's group in the hybrot (DeMarse et al., 2001; Bakkum et al., 2004, 2007). In their recent papers, robots or simulated robots were controlled by a new statistic, the centre of neural activity (CA) of responses within 100 ms after each electrical stimulus. These responses were evoked by a 'prove' stimulus applied to the neural network every five seconds. 'Prove stimuli' mimic the sensory inputs of animals, which are continuously derived from the brain. Potter et al. used well-designed stimulation protocols and ensured that the neuronal network remained stable. Interestingly, they also succeeded in goal-directed learning (Chao et al., 2008).

Our approach in the neuro-robot is different from their original concept in the following points.

1 The LNN is the only decision maker of the system. The system does not include any additional supervisor for generating behaviours.

2 Instead of continuous prove stimuli, the neuronal network is stimulated only when actual sensors are activated.

3 We do not discriminate between spontaneous activity and activity evoked by sensor inputs. We treat the spontaneous activity as a representation of spontaneous behaviour or 'thinking'.

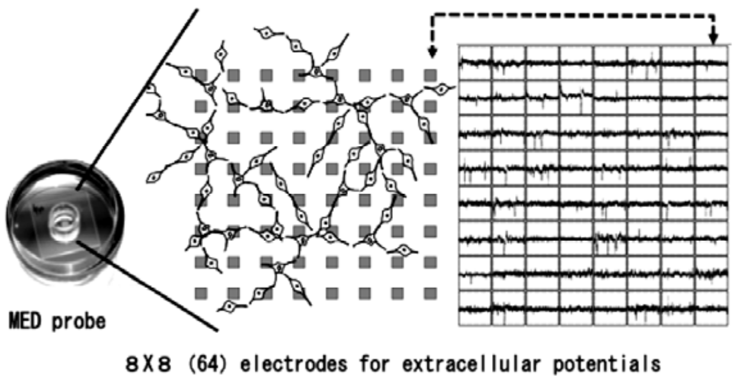
Here, we propose that this type of 'closed-loop' interaction between the LNN and the outer world, by which information from the outer world reflects not only on modification of synaptic strength but also the fluctuated internal state of the neuronal network. We call the neuro-robot system 'vitroid', because it utilises the fluctuated states of the LNN *in vitro*. In this study, we used a Khepera II robot for the body of the system and succeeded in eliciting obstacle avoidance behaviour with premised control rule sets.

2 System integration of vitroid

2.1 Preparation of the LNN

In the vitroid system, the central processing unit is an LNN cultured on a special culture dish embedded with an array of planar microelectrodes (Gross et al., 1977, 1982; Pine et al., 1980; Jimbo et al., 1993). Methods for preparing an LNN from rat hippocampus (a specific region of the brain, which is concerned with memory formation) have been described in previous papers (Kudoh et al., 1997; Kudoh and Taguchi, 2003). Our method is a modified conventional Banker and Cowan's method (1977). Briefly, the hippocampus was dissected from Wistar rats on embryonic day 17 (E17) or E18 and neurons were dissociated with 0.175% trypsin (Invitrogen-Gibco, USA) in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS-minus, Nissui) supplemented with 10 mM glucose at 37°C for 10 min. Neurons were then plated on a MED probe (Alpha MED Science, Japan), a culture dish with 64 planar microelectrodes on the bottom of the dish (Oka et al., 1999). The inter-electrode distance is 455 μm. The MED probe was pre-coated with 0.02% polyethyleneimine overnight. Neurons were seeded in the cloning ling put on the centre of the MED probe. The density of the seeded cells was 7,800 cells/mm^2. The culture medium was a mixture of Dulbecco's modified minimum essential medium (Invitrogen-Gibco, USA) and Ham's F12 (Invitrogen-Gibco, USA), supplemented with 5% horse serum and 5% fetal bovine serum. Half of the culture medium was exchanged with fresh medium every second day. The neurons were cultured at 37°C in 5% CO_2/95% air at saturating humidity. All experimental procedures were conducted according to the *Guidelines for the Care and Use of Laboratory Animals* of the AIST.

Figure 2 Scheme of electrical recording by the MED system

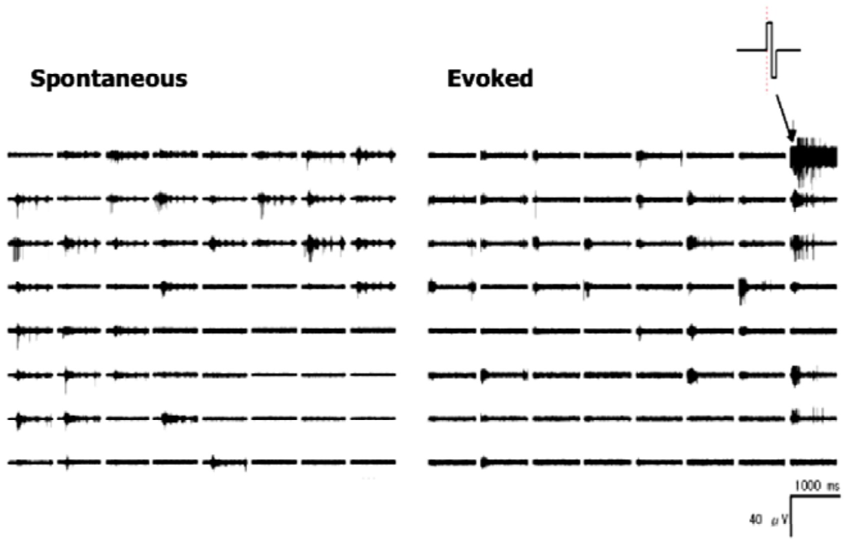


Dissociated neurons elongated neurites and re-constructed a synaptically connected network on the MED probe. An example of an LNN is indicated in Figure 1. Arrowheads indicate the planar microelectrodes of the MED probe. An arrow indicates a representation of the neurons. The bar equals 50 μm. In our culture conditions, the density of cells is high compared to conventional neuron cultures, but low compared to the density in the animal brain. 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.

Spontaneous extra-cellular action potentials were often observed in the normal culture medium by approximately 20 to 60 days *in vitro* (DIV) without any external input. The extra-cellular potentials were recorded through 64 electrodes simultaneously using the integrated MED64 system (Alpha MED Science, Japan) at a sampling rate of 10 kHz (Figure 2).

Action potentials evoked by current stimulation can also be recorded by the system. Figure 3 shows examples of spontaneous and evoked action potentials observed at each electrode.

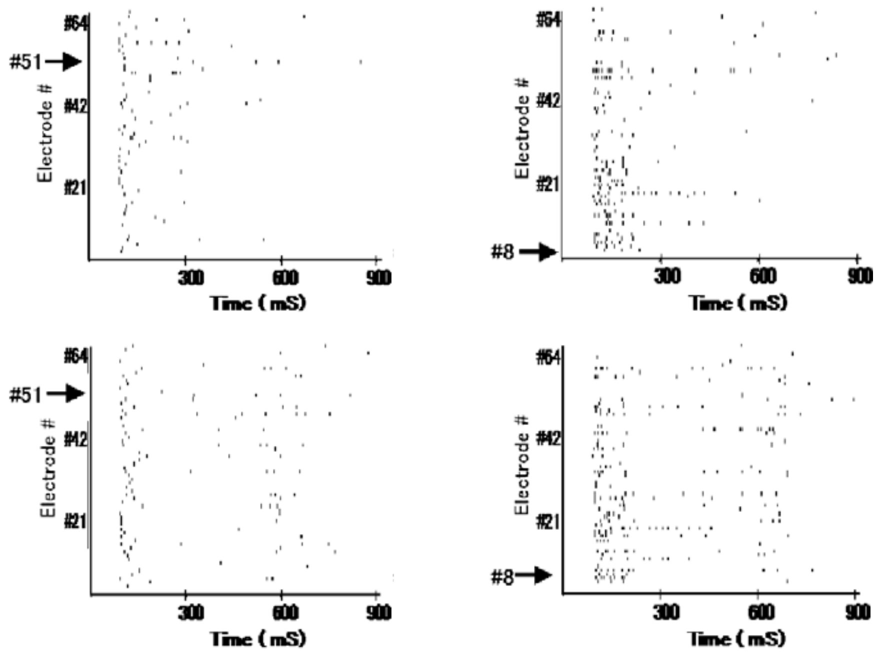
Figure 3 Examples of spontaneous and evoked action potentials observed at each electrode (see online version for colours)



The stimulation electrode is indicated by the arrow. The spikes of action potentials were detected automatically using an amplitude threshold-based algorithm for detection. The threshold was set to be three fold the level of baseline noise during 50 msec time windows. Extra large spikes of stimulation artefacts were omitted. A spike-sorting procedure was not performed in this study (Eytan and Marom, 2006), because only a small number of neurons were sensed by a single electrode, and the spike-sorting procedure requires a great deal of computational cost, preventing real-time processing in the control robot.

Figure 4 shows an example of the spatiotemporal pattern of detected action potentials. The vertical axis indicates the electrode numbers and the horizontal axis indicates the time. Each dot in the graph indicates the time stamp of the evoked action potential. Left panels indicate the pattern evoked by electrical stimulation of the #51 electrode, and the right panels indicate the pattern evoked by stimulation of the #8 electrode. The spatiotemporal pattern of action potentials evoked by the same stimulation (the same stimulation electrode) were very similar (Figure 4), suggesting that the LNN was able to discriminate several patterns of evoked action potentials according to different input sites. The LNN can express several patterns independently, corresponding to the observation that there are discriminated symbols in the animal brain evoked by sensory inputs from the outer world. The responses of the cultured LNN to the same stimulation protocol and stimulation sites are not completely identical. In particular, the patterns of late peak activity differ. We designed the vitroid to interpret such fluctuations of the LNN as the computational result of the LNN.

Figure 4 An example of the spatiotemporal pattern of detected action potentials



2.2 *Embodiment of vitroid*

We used a Khepera II robot (K-team) or a robot constructed using the LEGO mindstorm NXT kit as the substantial body of the vitroid. In both vitroid robot bodies, infra-red (IR) sensors were used for the ‘eyes’ of the vitroid for detecting obstacles. Two actuators of the vitroid body corresponded to the ‘legs’ of the vitroid. The movement of the vitroid was controlled by modulation of the balance of the left and right actuator speeds. In the case of animals, embodiment is completely provided by evolution. All actuators (muscles) and sensors are connected correctly and the relationships between them offer suitable responses to environmental inputs. In vitroid, such genetic relationships between neurons and actuators or sensors are abolished by removing the neurons from their body and dissociating them. It is the role of the experiment or to provide adequate connectivity between the re-organised neuronal network and the robot body (Figure 5).

This connectivity is expressed by the matching rules of the spatiotemporal pattern of the neuronal activity and the desired behaviour of the robot body. The rules are described in the ‘input interpreter’ and ‘output interpreter’ control programmes, written by Lab VIEW (national instruments). The system consists of five independent programmes on two PCs (for distribution of computational costs) and a recording system for multiple-site extracellular potentials (Figure 6).

Figure 5 The vitroid embodiment scheme (see online version for colours)

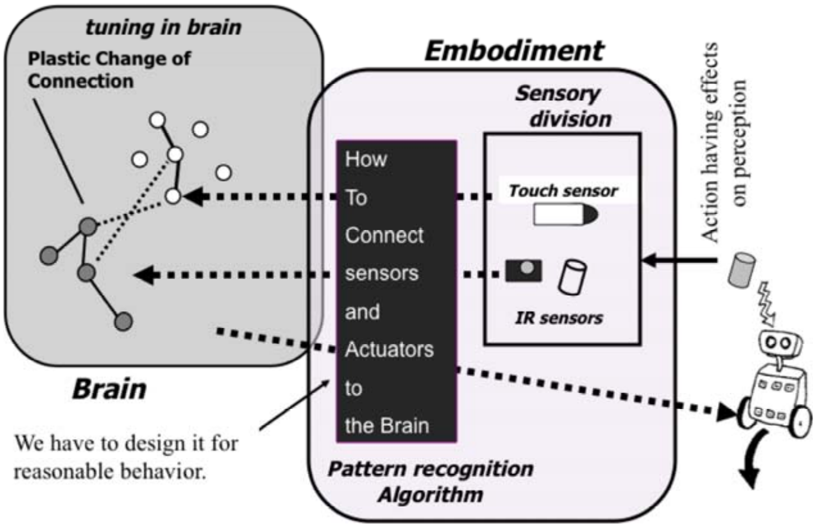
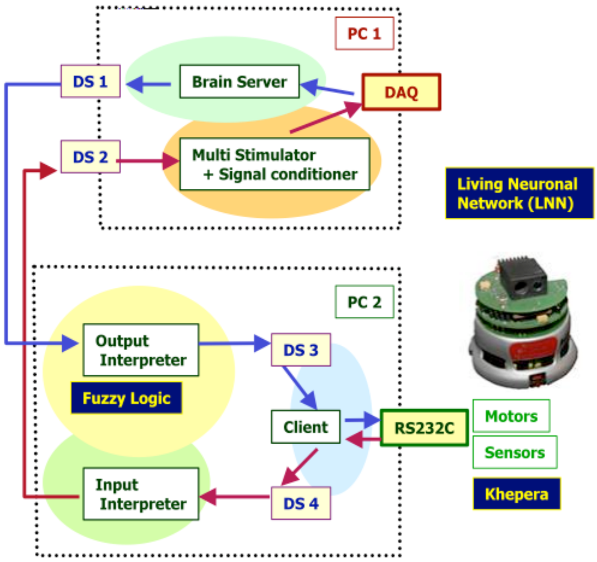


Figure 6 Scheme of vitroid, the neuro-robot system (see online version for colours)



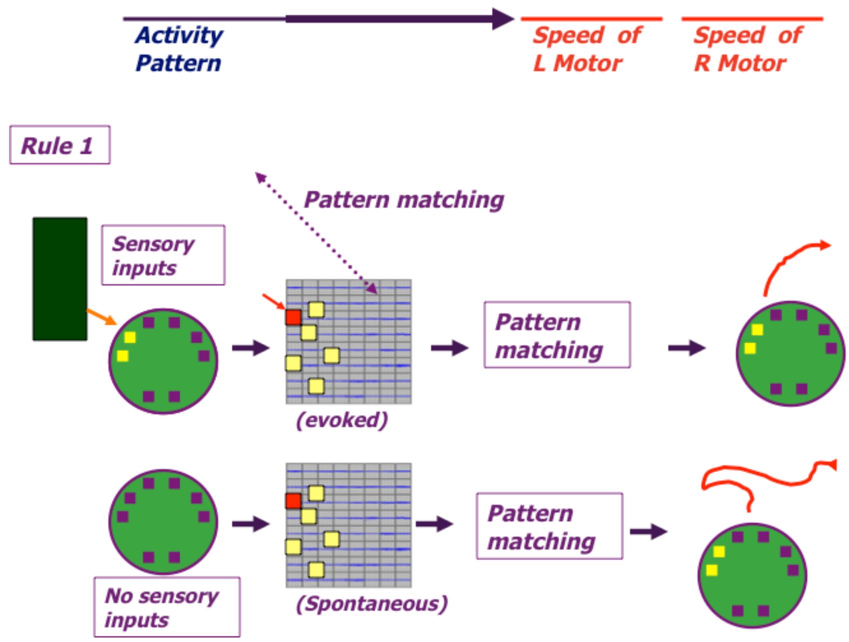
The 'brain server' programme records electrical potentials and detects action potentials of neurons associated with eight electrodes selected from the 64 electrode array. The system includes two interpreters: one is an 'output interpreter', translating the pattern of the electrical activity of the LNN into a specific behaviour of the robot; the other is an 'input interpreter', translating the sensory inputs from the robot body into stimulation patterns to the LNN. The 'client' programme controls the robot body. The 'multi stimulator with signal conditioner' programme stimulates the LNN, according to stimulation commands generated by the input stimulator. Programmes exchange processing data information mediated by a datasocket transfer protocol (DSTP, national instruments). The system uses four datasocket servers (National Instruments Inc., 2003) without buffering data, and there is a probability of lost data, but this system provides the second-best policy for avoiding increased time delays caused by data processing.

2.3 Input and output interpreters

The input interpreter translates in puts from the six IR sensors of the Khepera robot into an electrical stimulation pattern. The stimulation pattern is generated by a simple method. The input interpreter compares the value of the IR sensors on the left and right side. For example, the system applies stimulation current to the previously selected stimulation electrodes for an R signal when the difference between the summations of the value of IR sensors on the right side and left side of the robot body exceeds the defined threshold.

The system determines the speeds of two actuators using an output interpreter algorithm according to the spatiotemporal pattern of the electrical activity of the neurons. There are many candidates for such a translation algorithm, and we adopted a self-tuning fuzzy reasoning approach in this study. The particular relationships between network activity and outer phenomenon were formed by a control rule set of electrical stimulation to the neuronal network, responding to outer phenomenon. Using these two interpreters, instinctive premised behaviour can be embedded into the vitroid system (Figure 7).

Figure 7 Collision avoidance behaviour of vitroid (see online version for colours)

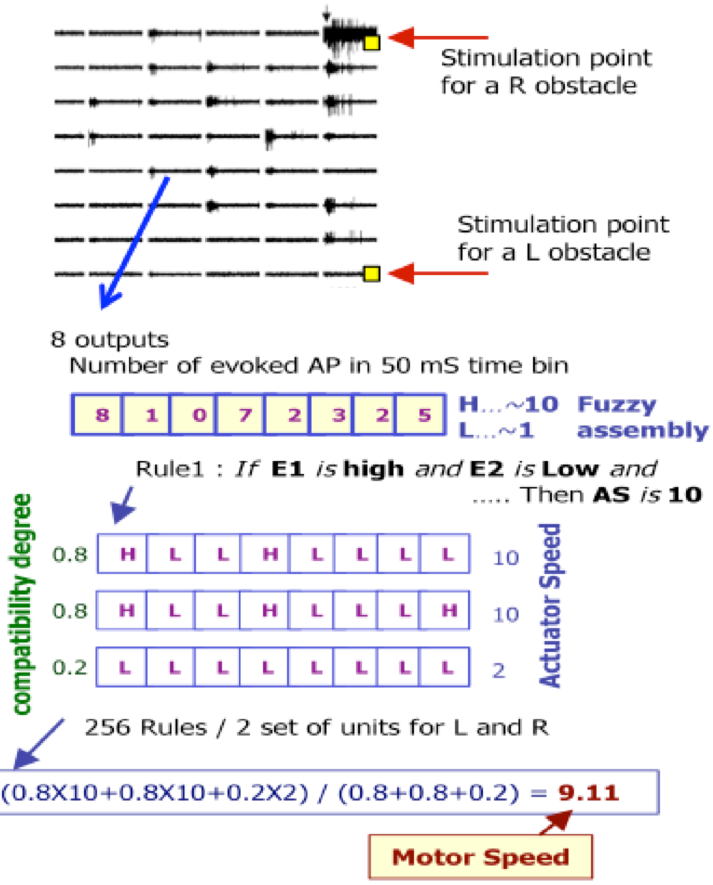


First, the particular patterns of the sensor values of the robot are translated in a stimulation pattern. This stimulation evokes a particular pattern in the LNN, according to that sensor's inputs. The system then recognises this pattern and controls the speed of the actuators to generate the appropriate behaviour by the robot body. For the example in Figure 7, IR sensors detect an obstacle at the left side, so the robot should increase the actuator speed in order to turn to the right. Where sensor input is absent, spontaneous electrical activity is generated only by the spontaneous internal state of the LNN, in that scheme, vitroid can also generate autonomous, voluntary behaviour.

2.4 *Simplified fuzzy reasoning implemented in the output interpreter*

The output interpreter uses two parallel sets of 256 fuzzy rules (Mamdani, 1974). The two sets of fuzzy rules have a common set of 256 antecedent clauses (if-part) and two distinct sets of 256 consequent clauses (Figure 8). The output interpreter receives eight signals from the LNN. Each signal is the number of detected action potentials per 50 ms time window from eight electrodes. Each input of the fuzzy reasoning unit has two types of fuzzy labels, high-frequency and low-frequency. The 256 fuzzy rules are constituted by eight inputs with high-frequency and low-frequency fuzzy labels. The use of 256 fuzzy rules is an over-specification for only two recognition states, but this large number of rules is used in order to describe all the classified patterns of eight inputs. This degree of complexity is required for analysis of neuronal activity, rather than for control of the actuators.

Figure 8 Pattern recognition by simplified fuzzy reasoning implemented in the output interpreter (see online version for colours)



The maximum frequency of the 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. For simplification, each membership function for all eight inputs is the same function currently. The common 256 antecedent clauses are used as pattern templates in the output interpreter. Inputted patterns are compared to these templates, and the compatibility degrees are calculated according to the similarities between the inputted pattern and each template. The degree of compatibility of each rule (template) is large when the spatial pattern of evoked action potentials is similar to the template. A motor speed value is then decided as a weighted average of the value of consequent clause (then-part) of each fuzzy rule, according to the following equation:

$$h_n = \prod_{k=1}^{8} \mu_{Ak}\left(\chi_k\right) \tag{1}$$

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

where z_o represents the output value of fuzzy reasoning, z_n represents the value of the consequent clause of each rule, h_n represents a compatibility degree for each rule, μ_{Ak} represents the fuzzy number of each input in each rule, and χ_k represents each inputted value to fuzzy reasoning. An inputted pattern of evoked action potentials has no relationship to the speed of an actuator in the vitroid. The rules also generate spontaneous behaviours in the vitroid. Spontaneous neuronal activity is classified into particular template patterns in the same manner, and the vitroid moves spontaneously.

To determine the quantitative relationships between the features of the inputted patterns and the speeds of the actuators, we adjusted the consequent clauses of each fuzzy rule by teacher learning. The tuning of the consequent clauses by teacher learning is performed by minimisation of the differences between the teacher signal and the output value of each fuzzy reasoning pair. The learning unit generates three categories of stimulation signals (L or R or no stimulation) and the optimal speeds of the actuators are used as a teacher signal. Electrical stimulations are then applied to the LNN and the corresponding responses are gathered and inputted into a fuzzy reasoning unit. The output of the fuzzy reasoning is assessed and the value of the consequent of each rule is adjusted. This consequent tuning is performed by the following equation:

$$z_n = z_n + \tau \cdot h_n\left(z_t - z_o\right) \tag{3}$$

where for all learning trials $i = 1,2,\ldots,n$, z_n represents a value of the consequent clause, and h_n represents a compatibility of degree for each rule. τ represents the learning coefficient, which decides the quantity of the adjustment, z_t represents a teacher signal (target value) for the output of the fuzzy reasoning, and z_o represents the output value of the fuzzy reasoning. As a result, the values of the consequent clauses are adjusted primarily by the similarity between the inputted patterns and the target patterns. A target pattern refers to the pattern repeatedly evoked by a particular input and the most general pattern response to the input. Another dominant factor of adjustment is the independency of the inputted pattern against the stimulation category (L or R). Before a test-run, an output interpreter of vitroid is trained. A total of 150 trials of stimuli were applied to an LNN for learning of consequent clauses of fuzzy rules. The initial value of consequent clauses of each rule was set at five. Teacher signals corresponding to an obstacle in left side were set at ten for a left actuator and one for a right actuator. Teacher signals corresponding to obstacles on the right side were set at one for a left actuator and tem for a right actuator. Adjusted values of consequent clauses did not completely converge during 150 trials of learning, but we assigned a priority to avoiding damage to the neurons by repeated current stimuli.

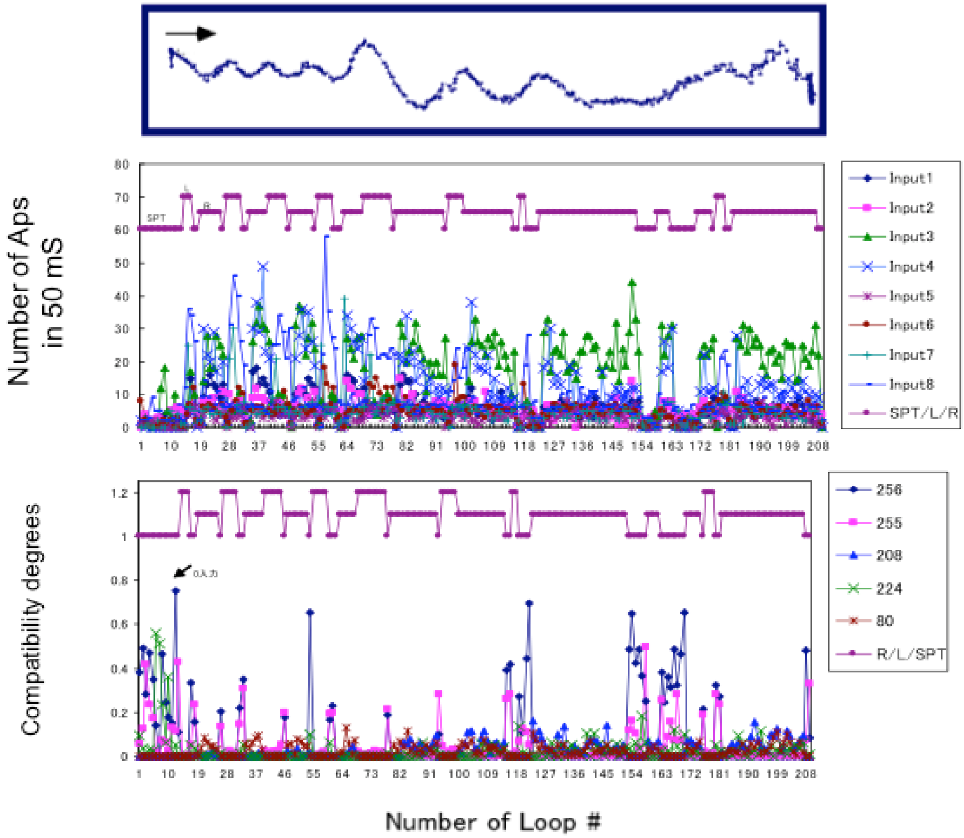
After the learning process, the parameters of the interpreters were fixed, meaning that the artificial part of the vitroid framework is not plastic. Thus, the plastic feature of the LNN may modify the matching between the LNN and the artificial framework, even though the LNN and its interpreters initially adapted to each other quite well. As a result, the direction of self-tuning of the LNN is often not suitable for reasonable behaviour, so we have to design an interface to match the reasonable behaviour and direction of self-tuning process of the LNN. In our paradigm, we do not perform conscious control of the LNN, instead we adapt the interface to the LNN.

3 Collision avoidance by vitroid

Figure 9 shows an example of the trajectory of the vitroid robot body (upper panel), the classified inputs (L, R,SPT) and number of inputs corresponding to the activities in eight electrodes (middle panel), and the compatibility degrees of the top five rules during an experiment. Vitroid succeeded in navigating a course between two parallel walls without any collision with a wall. In later experiments, it seemed that collision avoidance delayed gradually (Figure 9).

The delay of collision avoidance may be due to the delay of communication between each unit of the vitroid system. This problem should be improved. The responses of neurons to inputs from the outer world were relatively stable. Our current results suggest that inputs linked to the 'L-side obstacles' or the 'R-side obstacles' evoked reproducible patterns of electrical activity, although spontaneous autonomous activity varied during experiments. Compatibility degrees discriminated signals of the L-side obstacles from the R-side obstacles, but the combination pattern of compatibility degrees varied. These results indicate that a certain recognisable 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 by the spontaneous internal states of the network, which explains why the pattern evoked by electrical stimulation is not completely constant. This feature of the LNN is thought to be one of the fundamental characteristics of the animal brain that enables a flexible adaptation to the outer world. We are now analysing the mechanisms, using vitroid, of our interactions between neurons and the outer world. The vitroid system is a sort of 'test tube' for cognitive responses made by a living component.

Figure 9 An example of the behaviour of the vitroid robot body (see online version for colours)



4 Conclusions

- We have integrated a LNN and a robot body using interpreters. We call the neuro-robot system 'vitroid'. Vitroid has in vitro LNNs and frameworks to utilise fluctuations in the LNN.
- Vitroid succeeded in performing collision avoidance. The system is a good modelling platform to clarify interactions between living neurons and the outer world and assess the effects of the outer environment on the activity of the living neuronal system.

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