Acquisition of Logicality in Living Neuronal Networks and its Operation to Fuzzy Bio-Robot System

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***Abstract*— Brain-computer-interface has been come into the research limelight. Network dynamics of neurons strongly effects to a control of computer or machine in the outer world. Dissociated culture system with multi-electrode array is useful for elucidation of network dynamics of neurons. We have been investigating action potentials of rat hippocampal neurons cultured on the dish connecting with the outer robot. However, we don't exactly comprehend logicality of living neuronal networks. In this paper, we identify logicality of living neuronal networks with three electrodes in mult-electrode array using fuzzy connective operators consisting of $t-norm$ and $t-conorm$ operators, and we introduce a straight running of fuzzy bio-robot. We concluded that the logicality of living neuronal networks is dynamically changed to weak OR connection from strong AND connection. Additionally, by applying the fuzzy bio-robot to a straight running, we analyzed plasticity of living neuronal network connected to the robot, and we discussed regularity of logical potential response of the neuronal networks.**

I. Introduction

Recently, Brain-computer-interface, shortly BCI, has been come into the research limelight [1], [2]. As a fundamental study of BCI, potential response and dynamics of living neuronal networks have been discussed, e.g., in hippocampal neurons [3]. Dissociated culture system with multi-electrode array is fully useful for elucidation of network dynamics of neurons [4]. Kudoh et al. [5] have been investigating action potentials of rat hippocampal neurons cultured on the dish with multi-electrode array. Bettencourt et al. [6] identify a relationship between the three electrodes in the dissociated culture system with information entropy. However, they don't discuss logicality of living neuronal networks enough. We don't also exactly comprehend logicality of living neuronal networks in multi-electrodes array connected with the outer world.

In this paper, we formulate a new algorithm to acquire logical relationship between three electrodes with fuzzy connective operators, and identify the relationship by adjusting parameters of t-norm and t-conorm operators [7]. We additionally introduce an example of straight running of fuzzy bio-robot, and discuss plasticity of living neuronal network [8]–[12]. We define here fuzzy operator with Schweizer's $t-norm$ and $t-conorm$ operators [13], which represents logical operator, algebraic operator, bounded operator, drastic operator and so on. With the Schweizer's $t-norm$ and $t-conorm$ operators, we formulate a new algorithm to acquire logicality of electrodes in rat hippocampal neurons cultured on the dish.

Additionally, we introduce the fuzzy bio-robot system in an example of straight running [14], [15]. We use a Khepera II robot as outer machine connecting with living neuronal networks. We succeeded in straight running with fuzzy logic [16]. Through the example, we discussed plasticity of living neuronal networks by analyzing the membership values in the antecedent part of fuzzy rules. Especially, we could associate a spatial pattern of action potentials in multi-electrodes with a particular phenomenon in the outer robot. We concluded that regularity of neuronal responses can control the outer robot logically.

II. Neuron Culture and Multi-Electrode Array

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 [5]. 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.).

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) [4] 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 $(20-25°)$. 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.

III. Fuzzy Connective Operators

The fuzzy connective operators consists of $t-norm$ and $t-conorm$ operators. $t-norm$ T is a projective function expressed by $T(x,y)\ :\ [0,1]\times[0,1]\ \rightarrow\ [0,1]$, which satisfies four conditions, id est, boundary conditions,

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monotonicity, commutativity and associativity. $t-norm$ operator includes logical product, algebraic product, bounded product and drastic product. $t-conorm$ operator S is dual function of $t-norm$ operator, which is expressed by $S(x,y):[0,1]\times[0,1]\rightarrow[0,1]$, and also includes logical sum, algebraic sum, bounded sum and drastic sum.

On the other hand, many parametric $t-norm$ and $t-conorm$ operators have been proposed. By changing the values of parameter, the parametric fuzzy operator become equal to the drastic $t-norm$ to the drastic $t-conorm$. For example, the parametric fuzzy operator proposed by Schweizer [13] is expressed as follows:

$$\begin{aligned} T(x,y) &= 1-((1-x)^{p_n}+(1-y)^{p_n} \\ &\qquad -(1-x)^{p_n}(1-y)^{p_n})^{1/p_n} \quad (1) \\ S(x,y) &= (x^{p_c}+y^{p_c}-x^{p_c}y^{p_c})^{1/p_c} \quad (2) \end{aligned}$$

where, p_n and p_c are parameters.

By changing values of the parameter p_n and p_c, the Schweizer $t-norm$ and $t-conorm$ become equal to logical operator ($p=\infty$), algebraic operator ($p=1$) and drastic operator ($p=0$).

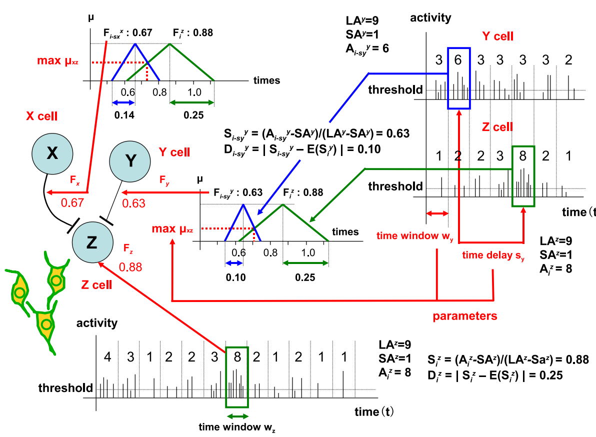

Fig. 1. Algorithm for Analysis of Action Potentials in Cultured Neuronal Network

IV. Acquisition of Logicality in Neuronal Networks

For acquiring logicality of neuronal networks, we detected action potentials of rat hippocampal neurons with multi-electrode array. The potentials data are provided as pulse-time series for $120s$ with $20Hz$ at 64 electrodes. We selected an arbitrary set of three electrodes x,y,z, and acquired the coherence pattern between the three electrodes. Now, we divide the data of pulse-time series in several time-windows, and set delay deviation between time-windows of input electrode and output electrode. The proposed algorithm is shown in Figure 1. In the electrode z as output elctrode, we define the pulse frequency, F_i^z, in the i-th time-window by the following membership function with center a_i^z and width c_i^z.

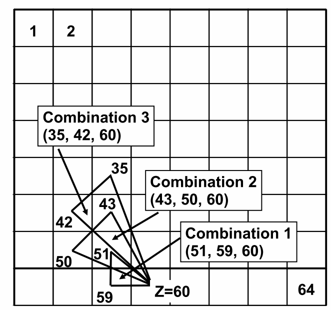

Fig. 2. Experiments

$$\begin{aligned} a_i^z &= \frac{p_i^z - sp^z}{lp^z - sp^z} \quad (3) \\ c_i^z &= |a_i^z - E(a_i^z)| \quad (4) \end{aligned}$$

where, p_i^z is the number of pulse in the i-th time-window, sp^z and lp^z are the minimum and maximum number of p_i^z, respectively. $E(a_i^z)$ is the average value of a_i^z.

The membership function $F_{i-s_x}^x$ with the delay deviation s_x is also defined in the electrode x as the input elctrode as same as the electrode z. Our purpose is here to let the degree of coincidence between F_i^z and $F_{i-s_x}^x$ maximize on the time x, where we denote the maximum degree of coincidence as μ_{xz}^*. To let the degree of coincidence maximize, the width of time-window w_x and the delay deviation s_x are adjusted. We denote the optimum pair of the width of time-window and the delay deviation by $Opt(w_x^*, s_x^*)$.

$$\begin{aligned} \mu_{xz} &= \sup_t \ \mu_{F_i^z}(t) \wedge \mu_{F_{i-s_x}^z}(t) \quad (5) \\ Opt(w_x^*, s_x^*) &= \max_{w_x, s_x} \ \mu_{xz}. \quad (6) \end{aligned}$$

We also optimize the pair of $Opt(w_y^*, s_y^*)$ in the electrode y and the electrode z.

Lastly, we calculate the output of the Schweizer operator with two inputs of the center $a_{i-s_x}^x$ and the center $a_{i-s_y}^y$ of membership function in the electrode x and y, respectively.

$$\begin{aligned} T(x,y) &= 1-((1-a_{i-s_x}^x)^{p_n}+(1-a_{i-s_y}^y)^{p_n} \\ &\qquad -(1-a_{i-s_x}^x)^{p_n}(1-a_{i-s_y}^y)^{p_n})^{1/p_n} \quad (7) \\ S(x,y) &= ((a_{i-s_x}^x)^{p_c}+(a_{i-s_y}^y)^{p_c} \\ &\qquad -(a_{i-s_x}^x)^{p_c}(a_{i-s_y}^y)^{p_c})^{1/p_c}. \quad (8) \end{aligned}$$

We then adjust the parameter p_n of $t-norm$ and parameter p_c of $t-conorm$ to minimize a deviation between the center a_i^z in the electrode z and Schweizer output, and select the optimum parameter p^* from either p_n or p_c to minimize the deviation.

$$p^* = \{p_n, p_c | \min_{p_n, p_c}(|T(x,y)-a_i^z|,\ |S(x,y)-a_i^z|)\}. \quad (9)$$

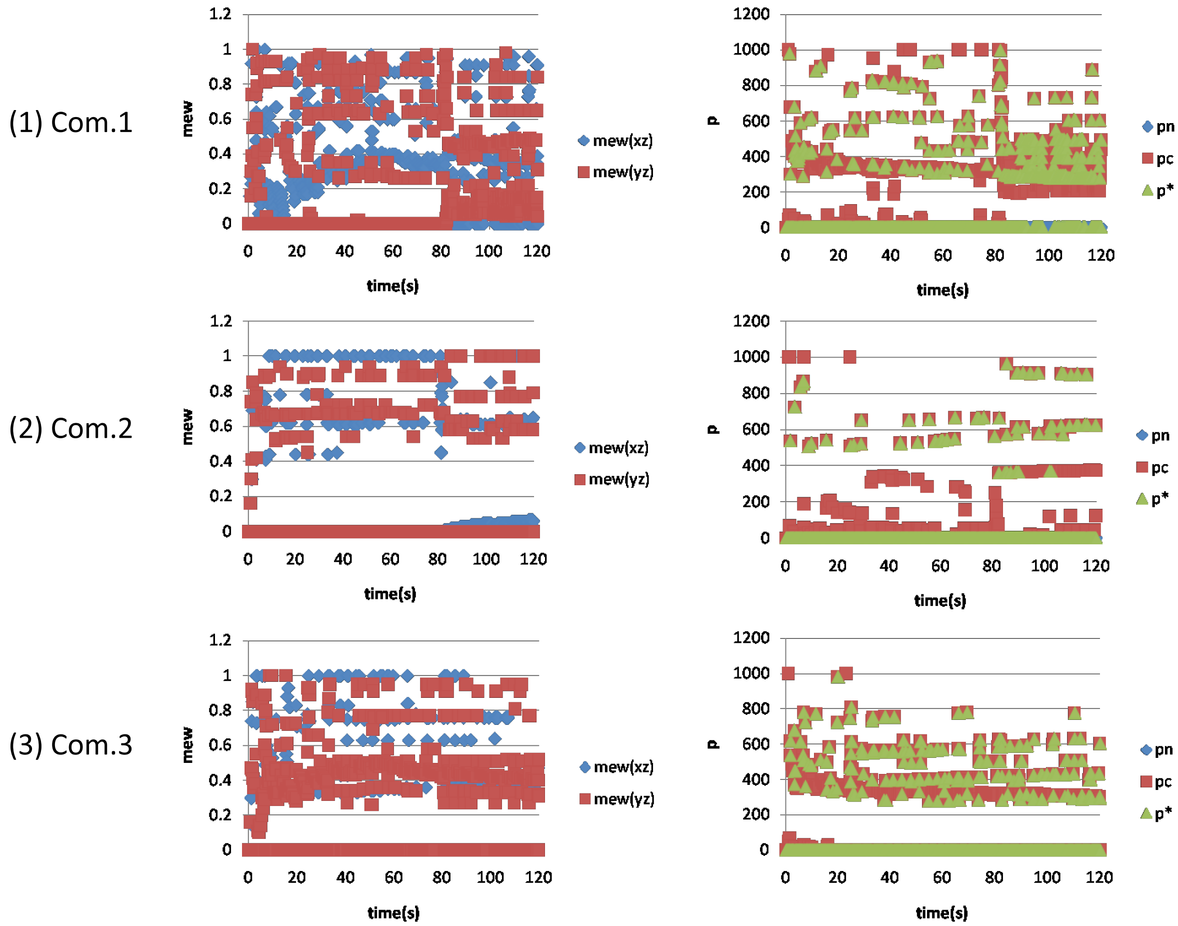

Fig. 3. Analysis of Action Potentials

For tangible data analysis, we selected three sets of input electrodes from 63 electrodes because of the output in the 60th electrode ($60el$). Figure 2 shows the location of three combinations of the input and output electrodes. In $60el$, we detected the sudden increasing of pulse frequency at $102.4s$ after the pulse frequency drastically decreased to 6 times at the around $95s$. For the analysis, we focus this characteristic pulse increasing at $102.4s$, and analyzed how this characteristic pulse influenced it for the following three combinations.

1. A combination of $(x, y, z) = (51el,\ 59el,\ 60el)$
2. A combination of $(x, y, z) = (43el,\ 50el,\ 60el)$
3. A combination of $(x, y, z) = (35el,\ 42el,\ 60el)$

The result is shown in Figure 3. We showed the degree of coincidence between membership functions μ^*_{xz} and μ^*_{yz}, and the optimum parameter values of fuzzy operators p^*, p_n and p_c. In the first combination of electrodes $(x, y, z) = (51el, 59el, 60el)$, the maximum degrees of coincidence are adjusted as $\mu^*_{xz} = 0.85$, $\mu^*_{yz} = 0.75$ with $w_x = 11s$, $w_y = 10s$, and the optimum parameter of Schweizer operator is converged to $p^* = p_c = 730.5$. In the second combination of electrodes $(x, y, z) = (43el, 50el, 60el)$, the maximum degrees of coincidence are adjusted $\mu^*_{xz} = 1.0$, $\mu^*_{yz} = 1.0$ with $w_x = 11s$, $w_y = 10s$, and the optimum parameter of Schweizer operator is converged to $p^* = p_c = 617.98$. In the third combination of electrodes $(x, y, z) = (35el, 42el, 60el)$, the maximum degrees of coincidence are adjusted as $\mu^*_{xz} = 0.76$, $\mu^*_{yz} = 0.91$ with $w_x = 11s$, $w_y = 10s$, and the optimum parameter of Schweizer operator is converged to $p^* = p_c = 630.23$. From these results, we conclude that the characteristic pulse increasing in $60el$ at $102.4s$ propagates to $(51el, 59el) \rightarrow (43el, 50el) \rightarrow (35el, 42el)$. And then, the parameters of Schweizer operator have been converged to $p^* = p_c = 730.5$ in $(51el, 59el)$, $p^* = p_c = 617.98$ in $(43el, 50el)$, and $p^* = p_c = 630.23$ in $(35el, 42el)$. These parameters mean logical sum. However, we should notice that the parameter of Schweizer operator at around $102.4s$ is $p^* = p_n = 0.0$, which means the drastic product. Therefore, despite all of our intuition, we conclude that the logicality of electrodes became to drastically change to weak OR relation from strong AND relation when a crowd of the pulses was fired and the pulse propagated distantly and widely.

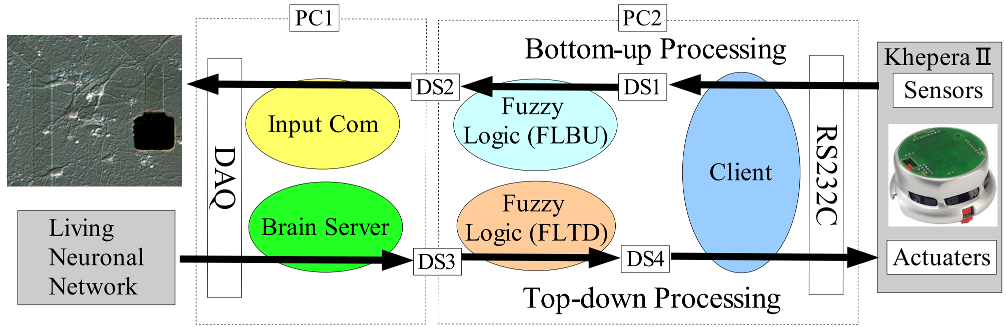

Fig. 4. Living Neuronal Network and Robot

V. Fuzzy Bio-Robot System

Fuzzy bio-robot includes two kinds of fuzzy logic units, that is FLTD and FLBU. The FLTD, Fuzzy Logic unit in Top Down, is located in top-down processing, and infers the rotation speed of robot actuator from the pattern of action potential in multi-electrode array. The FLBU, Fuzzy Logic unit in Bottom Up, is located in bottom-up processing, and infers the electrical stimulation points in multi-electrode array from output values of robot sensors. Figure 4 explains the relationship between living neuronal networks and robot, and Figure 5 shows the concept of fuzzy bio-robot system.

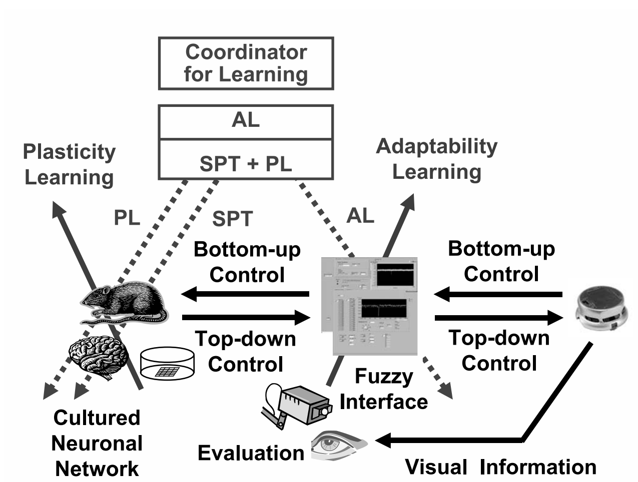

Fig. 5. Fuzzy Bio-Robot System

Figure 6 shows how to control a robot with living neuronal networks via fuzzy logic. We designed closed loop in which the robot of Khepera II receives the rotation speed of actuator in [-20, 20] from FLTD for eight inputs of patterns in multi-electrode array. Additionally, the multi-electrode unit receives stimulation points from FLBU for eight IR sensors of the robot. We designed 256 fuzzy rules with eight inputs and two output in FLBU and FLTD, respectively.

Now, we explain how to design fuzzy rules in FLTD. First, we divide 64 electrodes in eight parts as inputs for FLTD, and define two kinds of membership functions of

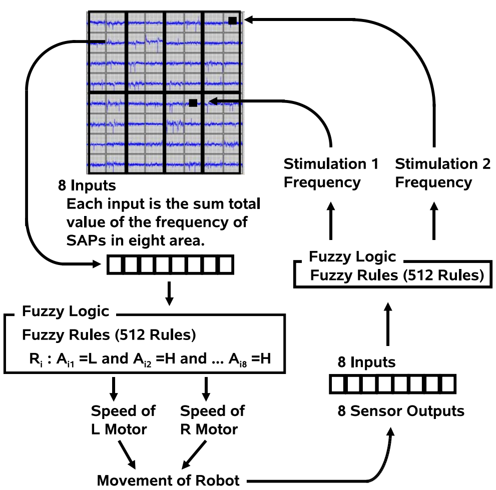

Fig. 6. Robot Control with Living Neuronal Network

"High" and "Low" potentials in each part of electrodes. Thus, we become to constitute 256 fuzzy rules. Two electrodes are arbitrarily seleceted as stimulus points, and we detect the potential response for the first stimulus from other 62 electrodes. The pulse pattern of potential responses is input to the antecedent part of fuzzy rules, and the membership value of each rule is calculated. Next, we detect the pulse pattern of potential responses for the second stimulus, and also calculate the membership value of each rules. For two different membership values, we calculate the subtraction between them and assign motor speed of robot actuators to rules whose differentials are large. We additionally adjust the value of motor speed better with the steepest descent method. If the neuronal networks have regularity of logical potential response, the robot will be controlled well.

To demonstrate the regularity of neuronal networks, we applied the fuzzy bio-robot system to the straight running. We estimate if the Khepera robot can run straight in a track without bumping into a wall. The running track is the length of 120mm and the width of 90mm. Figure 7 shows the running track.

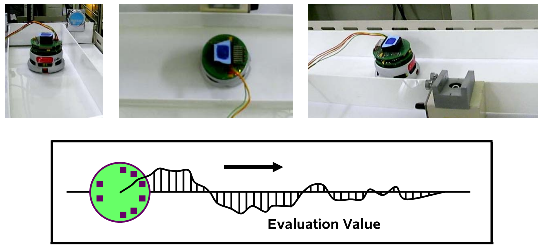

Fig. 7. Experimental Course

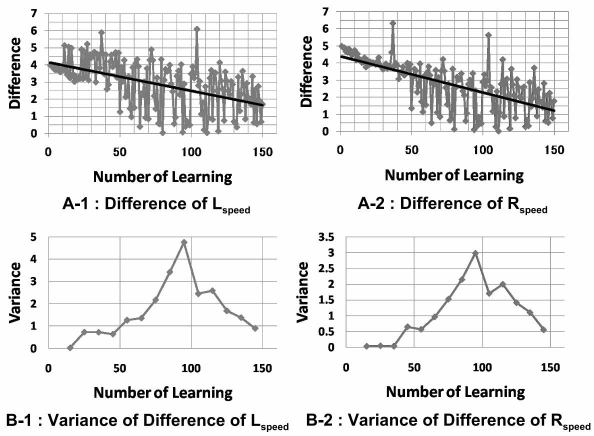

Fig. 8. Learning of Fuzzy Rule

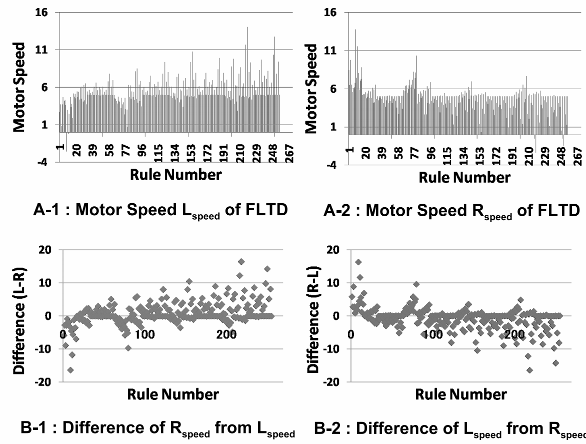

Fig. 9. Consequent Motor Speed of Fuzzy Rule

The deviation between the output of FLTD and target output is shown in the part A of Figure 8. The variance of each 10 times of learning is shown in the part B. The deviation is gradually decreasing according to the number of learning. Actually, the deviation of the left actuator, L_{speed}, decreased by 40.3% for 50 learning times and become 1.673. The deviation of the right actuator, R_{speed}, decreased by 27.8% and become 1.224.

Next, we show the motor speed, the subtraction $L_{speed} - R_{speed}$ and $R_{speed} - L_{speed}$ of each fuzzy rule in Figure 9. We should notice that the control values to turn on the right are assigned to the consequent part of the higher number of fuzzy rules because of the large deviation of $L_{speed}-R_{speed}$. The control values to turn on the left should be also assigned to the consequent part of the lower number of fuzzy rules because of the large deviation of $R_{speed} - L_{speed}$.

We next monitored the membership value of fuzzy rules fired while the robot is running in a track under condition of $1mM$ density of Mg_2^+. Figure 10 shows a trajectory of the robot running and the membership values in FLTD. The part A shows the changing of sensor value detected with Khepeara. The part B shows the membership values in FLTD. We also figured the changing of membership value under condition of $5mM$ density of Mg_2^+ for comparison of $1mM$ density. The Khepera II robot could run in a straight without bumping on the wall. In the part A, we monitored two high frequency pulses of "Input 4" for "L Stimulus", and "Input3" for "R Stimulus". In the part B, we detected the high membership value in the 256th fuzzy rule whose membership functions are all "Low" potentials. That is, the Khepera robot is usually running in a straight with spontaneous action potentials with the 256th fuzzy rule, however the specific fuzzy rules to avoid collision with wall are fired when the Khepera was too close to wall.

To discuss the fuzzy rules to avoid collision with wall in more detail, we monitored fuzzy rules whose membership values are relatively higher until $40s$ in Figure 11. In the part A, the Khepera robot detects the wall in the left side, and turns on the right with the 13th and 14th fuzzy rules, or the 15th and 16th fuzzy rules, simultaneously. The specificity of these fuzzy rules pattern appears regularly. In other words, the neuronal networks have regularity of logical potential response.

Finally, we observed the trajectory of the Khepera with camera placed above of the track course. We image a base line drawning along the centerline of the track course from the start position of the Khepera. We detected the deviation between the base line and the trajectory of the Khepera, and defined absolute value of the deviation as evaluation value. Figure 12 shows a change of the evaluation for running trials of iteration. The evaluation values decrease as running trials of iteration, and the Khepera became to run along the base line. We conclude that the decreasing will be due mainly to plasticity of neuronal networks.

In addition, we calculated the rate of the Khepera completed the course in 20 trials in Table I. The completed courses were 16 times, and the case of the Khepera crashed on the wall and stopped were four times. However, the rate of completed the course is high with 80%. We conclude that the logicality of neuronal networks and the adaptability of the fuzzy logic work efficiently. We should also conclude that the rate 80% is extremely high because of living neuronal networks.

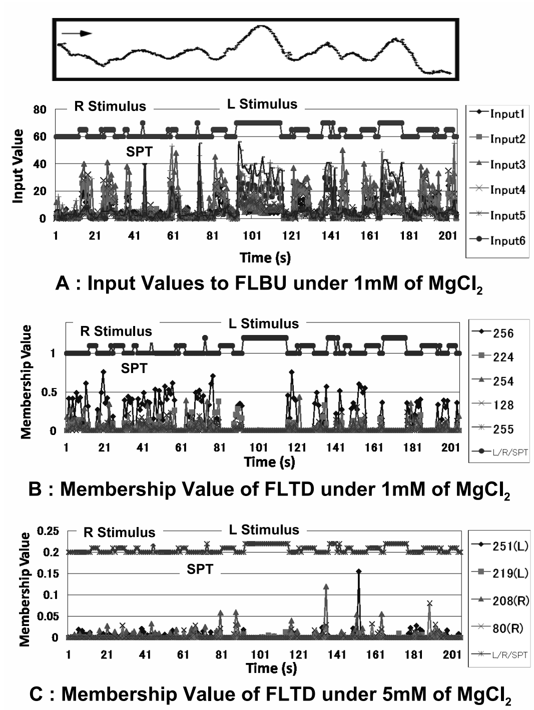

Fig. 10. Trace of Khepera Robot and Membership Values of FLTD

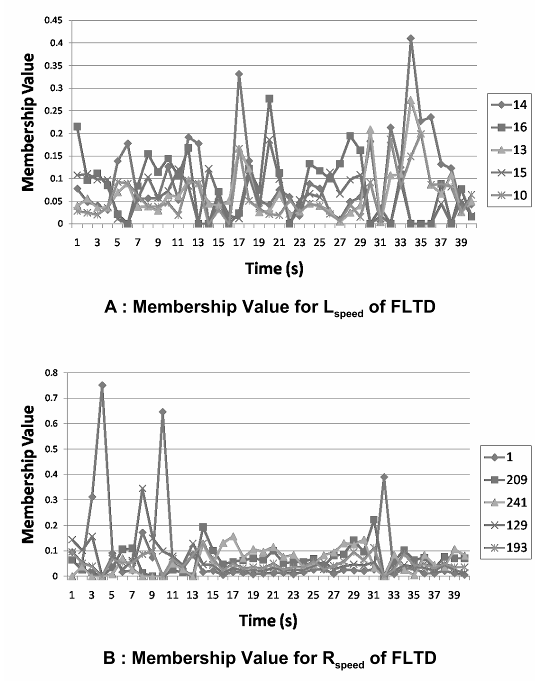

Fig. 11. Membership Values of FLTD until 40s

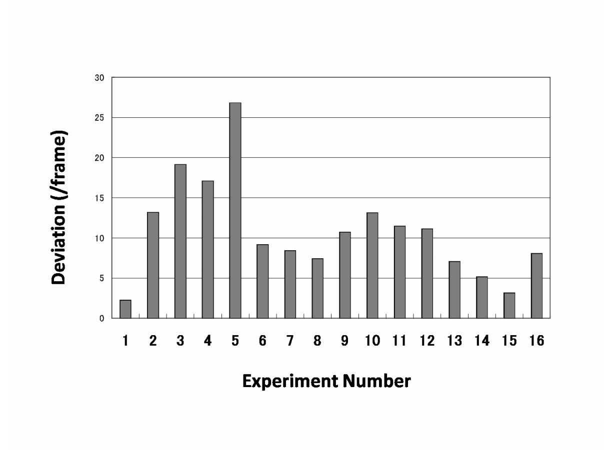

Fig. 12. Trace Deviation of Khepera Robot

TABLE I
COMPLETED RUN RATE OF KHEPERA ROBOT

	Experiment Number	Rate
Completed Run	1,3,4,5,6,7,8,9,10,14 15,16,17,18,19,20	80.0%
Incompleted Run	2,11,12,13	20.0%

VI. CONCLUSION

In this paper, we formulated a new algorithm to acquire logical relationship between three electrodes with fuzzy connective operators. We also introduced an example of straight running of fuzzy bio-robot, and discussed plasticity of living neuronal network connected to the robot. We should discuss the relationship between plasticity of living neuronal networks and adaptability of fuzzy logic more deeply in the near future.

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