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Description of Activity of Living Neuronal Network by Fuzzy Bio-Indicator

Isao Hayashi
Department of Informatics, Kansai University
Takatsuki, Osaka 569–1095, Japan
Email: ihaya@cbii.kutc.kansai-u.ac.jp

Suguru N. Kudoh
Kwansei Gakuin University
Gakuen, Sanda, Hyogo, 669–1337, Japan
Email: snkudoh@kwansei.ac.jp

***Abstract*—The culture dish describes the small fundamental world resembling human brain function. Multi-site recording system for extracellular action potentials is used for recording the activity of living neuronal networks. The living neuronal network is able to express several patterns independently, and that's meaning that it has fundamental mechanisms for intelligent information processing. In this paper, we propose a model to analyse logicality of signals and connectivity of electrodes in a culture dish of rat hippocampal neurons. We call it "fuzzy bio-indicator". This indicator is a kind of mapping methods to show logicality and connectivity of pulse frequency from active potential of neuronal network. We try to analyze the dynamics of action potentials of neuronal networks by the fuzzy bio-indicator, and identify the logicality and connectivity of neuronal networks through the indicator. We show here the usefulness of fuzzy bio-indicator through numerical examples and action potential detected from the culture neuronal network.**

***Index Terms*—Living Neuronal Network, Culture System, Fuzzy Connectives.**

I. Introduction

The culture dish describes the small fundamental world resembling human brain function [1]–[3]. The rat hippocampal neurons are organized into complex networks in a culture dish with 64 planar microelectrodes. The living neuronal network is able to express several patterns independently, and that's meaning that it has fundamental mechanisms for intelligent information processing [4], [5]. In particular, many models as to the rat hippocampal neurons which are organized in the culture dish have been discussed [6], [7]. Bettencourt et al. [7] classify the logicality and the connectivity of action potentials of three electrodes on the multi-site recording system according to their entropies and have discussed the characteristic of each classification. However, they only discuss the static aspects of logicality and connectivity relations among the electrodes but not the dynamics of such connectivity concerning how the strength of electrode connection changes when a spike is fired. Therefore we try to analyze the dynamics of action potentials of living neuronal networks by a structure model, and identify the logicality and connectivity of living neuronal networks through the model. Under the condition where spontaneous action-potentials and evoked-action potentials in a culture dish are contained in signal asynchronously, fuzzy model needs in order to describe pulse rule [8]–[10].

In this paper, we propose a model to analyse logicality of signals and connectivity of electrodes in a culture dish of rat hippocampal neurons [11], [12]. We call it "fuzzy bio-indicator". This indicator is a kind of mapping methods to show logicality and connectivity of pulse frequency from active potential of neuronal network. We're not sure that that system is unique, however, we discuss here how to indicate the logicality and the connectivity of living neuronal network. First, we propose a new algorithm using parametric fuzzy connectives, that consist of both $t - norm$ and $t - conorm$ operators [13], [14], in order to analyse the logicality and the connectivity of those three electrodes. Next, we classify propagation patterns of pulse frequency to three formulations, which are transmission, diffusion, and absorption, and we define an evaluation of fuzzy inclusion degree as corresponding of pulse frequency between electrodes for each propagation pattern. Finally, we plot the activity of pulse frequency in figures as the fuzzy bio-indicator. As a result, the logicality of neuronal network in the culture dish is described with fuzzy connectives that consist of $t-norm$ and $t-conorm$, and the connectivity is described with fuzzy inclusion degree for each propagation pattern. We show here the usefulness of fuzzy bio-indicator through numerical examples and action potential detected from culture neuronal network.

II. Neuron Culture and Multielectrode 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 [4]. 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 10-100 days after the start of the culture. The spontaneous action potentials (sAPs) were gathered with the MED64 system (Alpha MED Sciences, Japan) 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 devel-

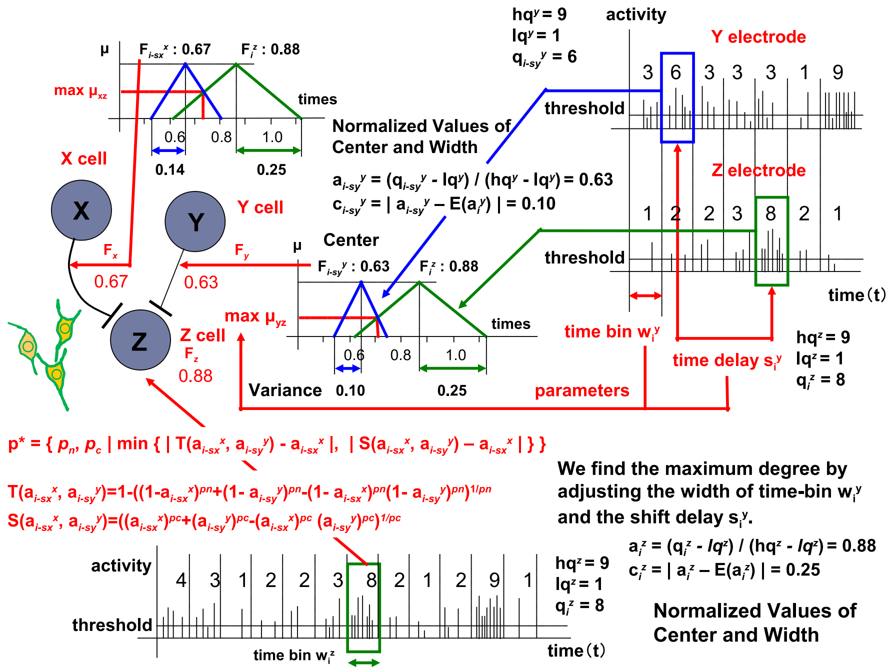

Fig. 1. Algorithm for Analysis of Action Potentials in Cultured Neuronal Network

oping program, sorted and classified by the amplitude versus decay time distributions using k-means cluster cutting method and converted to event trains.

III. Logicality of Neuronal Network

Fuzzy connective operators consist of $t-norm$ and $t-conorm$ operators. The $t-norm$ T is a projective function expressed by $T(x,y) : [0,1] \times [0,1] \rightarrow [0,1]$, which satisfies four conditions, boundary conditions, monotonicity, commutativity and associativity. The $t-norm$ operator T includes logical product, algebraic product, bounded product and drastic product. The $t-conorm$ operator S is a dual function of the $t-norm$ operator, which is expressed by $S(x,y) : [0,1] \times [0,1] \rightarrow [0,1]$, and 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 values of parameter, a parametric fuzzy operator expresses any operator between the drastic $t-norm$ and the drastic $t-conorm$. For example, the parametric fuzzy operator proposed by Schweizer [13] is expressed as follows:

$$T(x,y) = 1-((1-x)^{p_n}+(1-y)^{p_n} -(1-x)^{p_n}(1-y)^{p_n})^{1/p_n} \quad (1)$$

$$S(x,y) = (x^{p_c}+y^{p_c}-x^{p_c}y^{p_c})^{1/p_c} \quad (2)$$

where, p_n and p_c are parameters.

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

By the Schweizer operator, $t-norm$ and $t-conorm$, we formulate a new algorithm for analysing the logicality and the connectivity of the living neuronal networks. Now, we selected an arbitrary set of three electrodes x, y, z, and analysed a coherence pattern between three electrodes. First, we distribute a data set of pulse-time series in several time-bins, and define a time-delay between time-bins of two electrodes. The proposed algorithm is shown in Figure 1. For the electrode z, we shape a fuzzy set of the pulse frequency, F_i^z, at the i-th time-bin by the following triangular membership function which has the center a_i^z and the width c_i^z.

$$a_i^z = \frac{q_i^z - lq^z}{hq^z - lq^z} \quad (3)$$

$$c_i^z = |a_i^z - E(a_i^z)| \quad (4)$$

where, q_i^z is the number of pulse at the i-th time-bin, lq^z and hq^z are the minimum and maximum number of q_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 s_x of the electrode x is shaped as same as the electrode z. Our purpose is to let the degree of coincidence, μ_{xz}^*, between F_i^z and $F_{i-s_x}^x$ maximize in the parametric conditions of the electrode x. To let the degree of coincidence maximize, the width of time-bin w_x and the delay s_x are changed widely.

$$\mu_{xz} = \sup_t \mu_{F_i^z}(t) \wedge \mu_{F_{i-s_x}^x}(t) \quad (5)$$

$$\mu_{xz}^* = \max_{w_x, s_x} \mu_{xz}. \quad (6)$$

We calculate μ_{yz}^* between the electrode y and the electrode z as same as the electrode x and the electrode z. By obtaining two couples of coincidence degrees, μ_{xz}^* and μ_{yz}^*, the connection of electrodes is figured as a kind of connectivities in Figures 2.

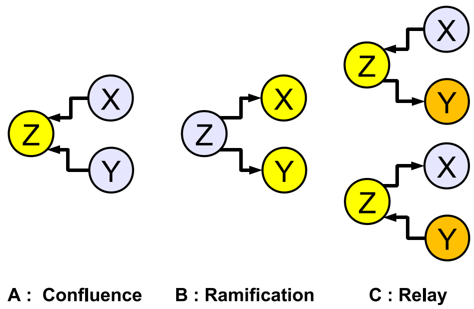

Fig. 2. Connectivity of Electrodes

Next, we calculate the output of the Schweizer operator with two centers of membership functions, $a^x_{i-s_x}$ of the electrode x and $a^y_{i-s_y}$ of the electrode y.

$$T(a^x_{i-s_x}, a^y_{i-s_y}) = 1 - ((1-a^x_{i-s_x})^{p_n} + (1-a^y_{i-s_y})^{p_n} - (1-a^x_{i-s_x})^{p_n}(1-a^y_{i-s_y})^{p_n})^{1/p_n} \quad (7)$$

$$S(a^x_{i-s_x}, a^y_{i-s_y}) = ((a^x_{i-s_x})^{p_c} + (a^y_{i-s_y})^{p_c} - (a^x_{i-s_x})^{p_c}(a^y_{i-s_y})^{p_c})^{1/p_c}. \quad (8)$$

We minimize the error deviation between the center a^z_i, and the Schweizer's output, $T(a^x_{i-s_x}, a^y_{i-s_y})$ and $S(a^x_{i-s_x}, a^y_{i-s_y})$, by changing the parameter p_n of $t-norm$ and p_c of $t-conorm$.

$$p^* = \arg\min_{p_n, p_c}(|T(a^x_{i-s_x}, a^y_{i-s_y}) - a^z_i|, |S(a^x_{i-s_x}, a^y_{i-s_y}) - a^z_i|). \quad (9)$$

The suitable parameter p^* represents the logicality of three electrodes. To illustrate the proposed algorithm, we show a simple numerical example. The spike frequency of three examples of electrodes x and z are shown in Figures 3 to 5, and Table I. At each example, we search a time-bin of electrode x which coincides most with the spike frequency of the sixth time-bin of electrode z. However, the "normalized order" of the horizontal axis in each figure normalized the number of spike frequency within each time bin as the same size. At the first example of Figure3, the spike frequency "2" of the fourth time-bin of electrode x coincided most with the spike frequency "2" of the sixth time-bin of electrode z with the degree $\mu^*_{xz} = 1.0$ of fuzzy sets. At the second and third examples of Figure 4 and Figure 5 respectively, the spike frequency "3" of the ninth time-bin of electrode x coincided most with the spike frequency "2" of the electrode z with $\mu^*_{xz} = 1.0$ as shown in Figure 4, and the spike frequency "1" of the sixth time-bin of electrode x coincided most with the the spike frequency "1" of the sixth time-bin of electrode z with $\mu^*_{xz} = 0.44$ as shown in Figure 5. We should notice that these results are understandable intuitively.

TABLE I
EXAMPLES OF ELECTRODE ANALYSIS

Time Bin	1	2	3	4	5	6	7	8	9	10
Example 1										
X	2	3	2	**2**	0	1	3	1	3	1
Z	0	0	1	3	1	**2**	0	0	0	0
CofX	0.67	1.0	0.67	**0.67**	0.0	0.33	1.0	0.33	1.0	0.33
WofX	0.07	0.4	0.07	**0.07**	0.6	0.27	0.4	0.27	0.4	0.27
CofZ						**0.33**				
WofZ						**0.27**				
μ	1.0	0.6	1.0	**1.0**	0.36	0.52	0.6	0.52	0.6	0.52
Example 2										
X	2	3	2	0	0	1	0	1	**3**	1
Z	0	0	1	0	0	**2**	0	0	0	1
CofX	0.67	1.0	0.67	0.0	0.0	0.33	0.0	0.33	**1.0**	0.33
WofX	0.23	0.57	0.23	0.43	0.43	0.1	0.43	0.1	**0.57**	0.1
CofZ						**0.33**				
WofZ						**0.8**				
μ	0.68	1.0	0.68	0.19	0.19	0.26	0.19	0.26	**1.0**	0.26
Example 3										
X	0	1	0	2	0	**1**	0	2	0	0
Z	0	0	1	2	3	**1**	0	0	0	0
CofX	0.0	0.5	0.0	0.1	0.0	**0.5**	0.0	0.1	0.0	0.0
WofX	0.3	0.2	0.3	0.7	0.3	**0.2**	0.3	0.7	0.3	0.3
CofZ						**0.33**				
WofZ						**0.1**				
μ	0.17	0.44	0.17	0.17	0.17	**0.44**	0.17	0.17	0.17	0.17

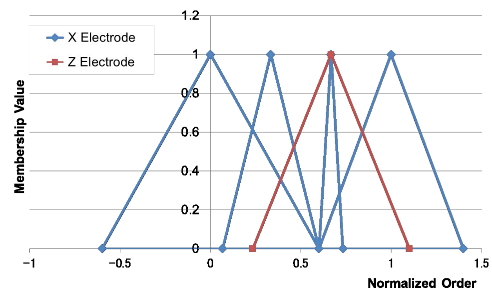

Fig. 3. Fuzzy Sets of the First Example of Electrode Analysis

IV. Connectivity of Neuronal Network

In order to discuss the connectivity of living neuronal network, we define propagation patterns of pulse fired at a electrode. We define an inclusion degree of fuzzy numbers as the connectivity of pulse frequency between electrodes for each propagation pattern. Thus, a characteristic of pulse frequency of living neuronal network is configured with logicality of $t-norm$ and $t-conorm$, and connectivity of this fuzzy numerical inclusion degree.

A propagation of the pulse frequency between electrodes is defined with three kinds of patterns, which are transmission, diffusion, and absorption as shown in Figure 6. The transmission shows a pattern that the pulse propagates to some direction, and the diffusion shows a pattern that the pulse propagates with spread directions. We call diffusion pattern with low angle a "diffusion-with-narrow-direction". We call diffusion pattern with high angle a "diffusion-with-wide-

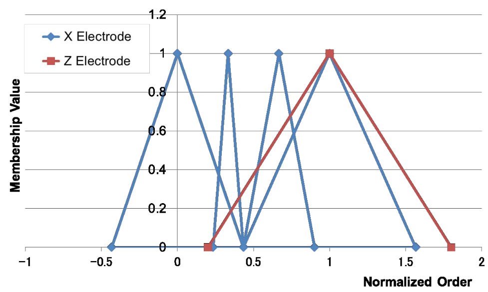

Fig. 4. Fuzzy Sets of the Second Example of Electrode Analysis

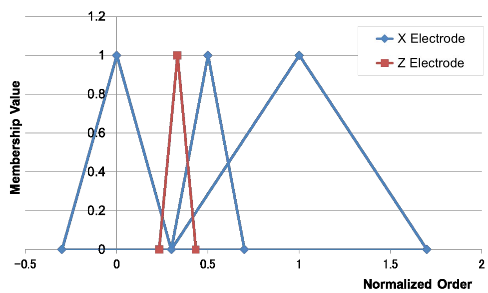

Fig. 5. Fuzzy Sets of the Third Example of Electrode Analysis

direction". We call diffusion pattern with whole direction a "diffusion-with-whole-direction". In addition, the absorption shows a pattern that the pulse propagates to some electrodes from wide direction. In Figure 6, (a) and (b) show an unidirectional transmission and an absorption respectively, and (c) and (d) describe diffusion.

For each propagation pattern, the connectivity of neuronal network is discussed with an inclusion degree of fuzzy numbers between electrodes. Figure 7 shows the inclusion degree of fuzzy numbers. The fuzzy inclusion degree means how degree of a fuzzy number of the pulse frequency is included by other pulse frequency. Now, the width of fuzzy number X, Y, and Z is described by D_X, D_Y, and D_Z respectively. A fuzzy inclusion degree γ of fuzzy number Z with fuzzy number X and Y is defined as follows;

$$\gamma = D_X/D_Z \times D_Y/D_Z \tag{10}$$

where, the degree γ get closer to one when the inclusion level of fuzzy numbers is high, and then the connectivity between electrodes is tight.

V. Indicator of Logicality and Connectivity

To indicate the logicality and connectivity of neuronal network, we should discuss delay time of pulse propagating to a neighbor electrode from a certain electrode. Since the propagation speed of a pulse is approximately $100m/s$, and distance between electrodes is $450\mu m$, the delay time between electrodes is $0.0045ms$. However, we estimated propagation time between electrodes at $10ms$ because the connection of neuronal network is not straight, and chemical neurotransmitter and synapse delay at the synapse have to be considered. In addition to parameters for analysing action potential, sampling frequency of the MED64 is 20kHz, and the time-bin is obtained at $4s$ in consideration of the overlap of time data. The number of time-bins is 30 because the measurement time is $120s$.

For analysing action potential in Figure 6, we set a couple consisting of the number of the electrode z and the time period of time-bin as follows;

$$\begin{aligned} a &: (19el,\ 44s - 48s) \\ b &: (3el,\ 88s - 92s),\ (4el,\ 88s - 92s),\ (11el,\ 88s - 92s) \\ c &: (31el,\ 88s - 92s) \\ d &: (39el,\ 80s - 84s). \end{aligned}$$

Figure 8 shows a result of transmission of pulse frequency, and Figure 9 shows a histogram in the transmission state. In Figure 8 the horizontal axis expresses parameter values p_n and p_c of fuzzy connectives, and the vertical axis shows the inclusion degree γ of fuzzy numbers. In Figure 9, the horizontal axis expresses parameter values p_n and p_c of fuzzy connectives. The vertical axes show the normalized frequency

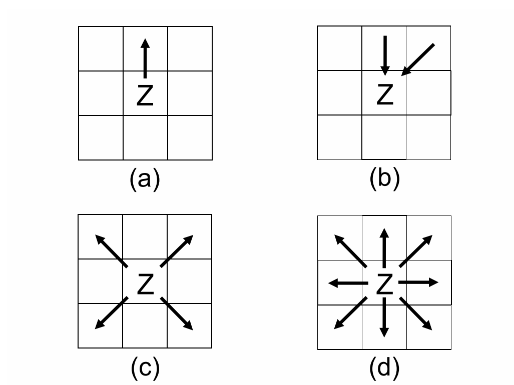

Fig. 6. Propagation Patterns

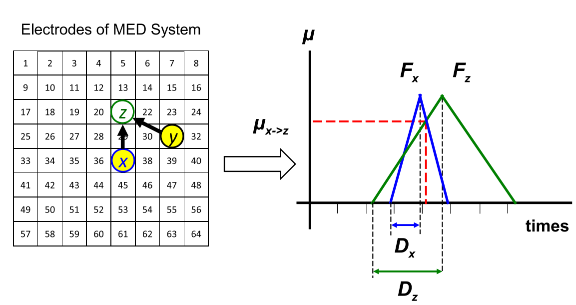

Fig. 7. Inclusion Degree of Fuzzy Numbers

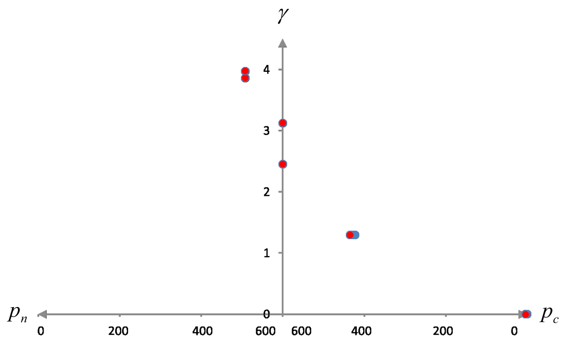

Fig. 8. Transmission of Pulse Frequency

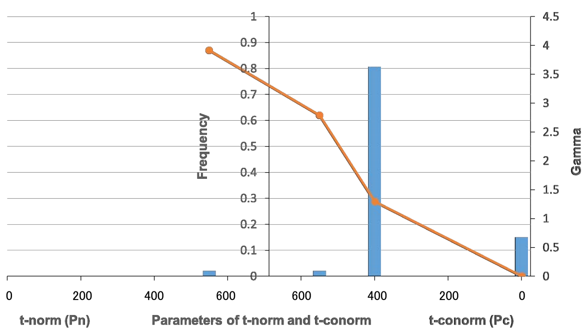

Fig. 9. Histogram in Transmission State

of fuzzy connectives as histogram format, and the average inclusion degree γ of fuzzy numbers as straight line. The 75 most suitable parameters exist near $\gamma = 1.29$ and $p_c = 430.0$. The 14 suitable parameters exist near $\gamma = 0.0$ and $p_c = 1.0$. In addition, the suitable parameters are distributed near $\gamma = 3.85$ and $p_n = 508.0$, and $\gamma = 3.97$ and $p_c = 508.0$. As a result, the most suitable parameters are located at $\gamma = 1.29$ and $p_c = 430.0$ from Figure 9. We should notice that the fuzzy connective operator is adjusted to a weak OR because the parameter $p_c = 430.0$ means the logical sum.

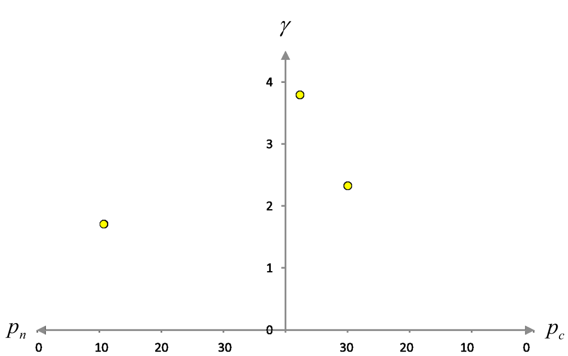

Fig. 10. Narrow Diffusion and Absorption of Pulse Frequency

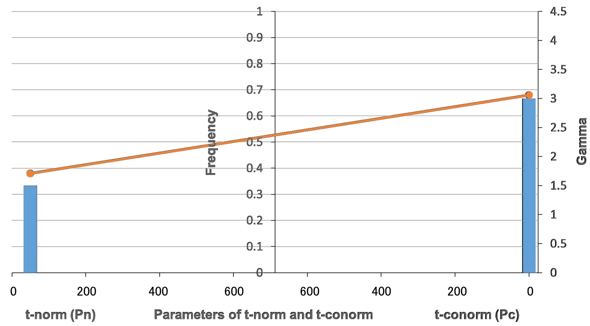

Fig. 11. Histogram in Narrow Diffusion and Absorption State

Figure 10 shows a result of diffusion-with-narrow-direction, and absorption of pulse frequency. Figure 11 shows the histogram. The most suitable parameters exist near $\gamma = 2.33$ and $p_c = 30.0$, and $\gamma = 3.79$ and $p_c = 37.7$. In addition, the suitable parameters exist near $\gamma = 1.72$ and $p_n = 10.7$, As a result, the most suitable parameters are located at $\gamma = 2.33$ and $p_c = 30.0$, and $\gamma = 3.79$ and $p_c = 37.7$ from Figure 11. We should notice that the fuzzy connective operator is adjusted to a weak OR relatively because the parameter $p_c = 30.0$ and $p_c = 37.7$ means the logical sum.

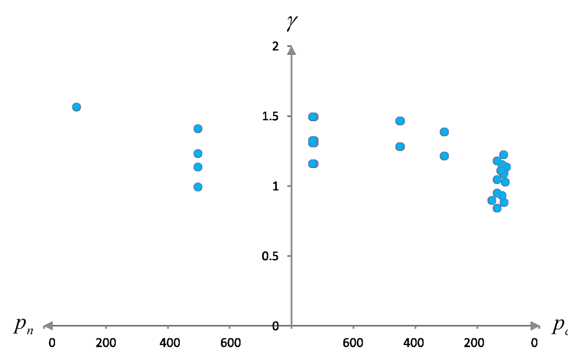

Fig. 12. Wide Diffusion of Pulse Frequency

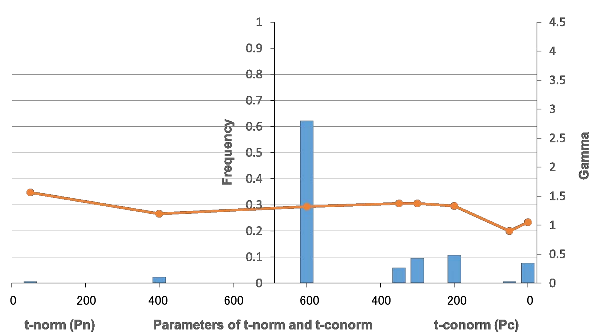

Fig. 13. Histogram in Wide Diffusion State

Figure 12 shows a result of diffusion-with-wide-direction of pulse frequency, and Figure 13 shows a histogram in the diffusion-with-wide-direction state. The degrees γ of many suitable parameters get closer to one, and then the connectivity between electrodes is tight. The 104 most suitable parameters exist near $p_c = 630.3$. The 13 suitable parameters exist near $p_c = 30.0$, the 18 suitable parameters exist near $p_c = 206.6$, and the 26 parameters exist near $p_c = 349.8$. The 4 suitable parameters exist near $p_n = 397.5$. In particular, the most suitable parameter is located at $p_c = 630.3$. We should notice that the fuzzy connective operator is adjusted to a weak OR because the parameter $p_c = 630.3$ means logical sum.

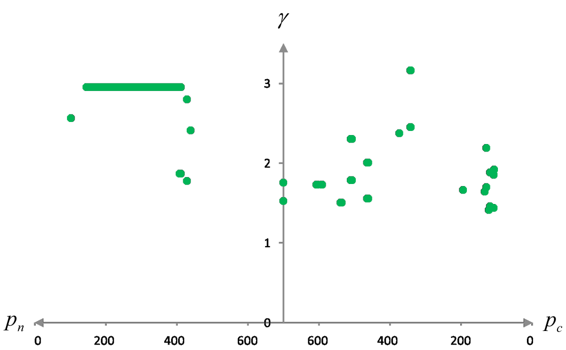

Fig. 14. Whole Diffusion of Pulse Frequency

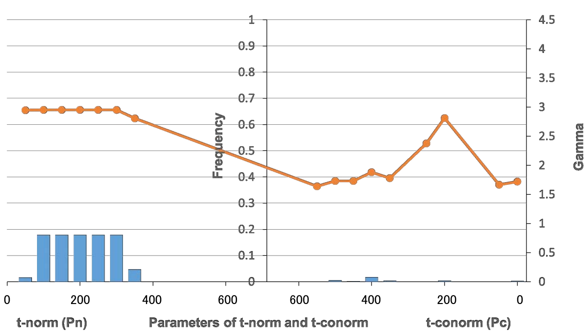

Fig. 15. Histogram in Whole Diffusion State

Figure 14 shows a result of diffusion-with-whole-direction of pulse frequency, and Figure 15 shows a histogram in the diffusion-with-whole-direction state. The suitable parameter spreads around $p_n = 200.0$. The fuzzy connective operator is adjusted to a weak AND because the parameter means logical product. However, the operator does not converge the specific values. That means that various kinds of the AND-logicality are mixed when the pulse frequency spreads in all directions.

From the overall result, we notice that the inclusion degree becomes higher and the fuzzy operator moves to the logical product from the logical sum, when the diffusion range is wide. In other words, the pulse is sure propagating without a loss of frequency when the propagation spreads with various kinds of operators widely.

VI. Conclusion

In this paper, we discussed how to indicate logicality and connectivity of living neuronal network with fuzzy connective operators and fuzzy inclusion degree. We should analyse the relationship between pulse of living neuronal networks and propagation pattern more deeply in the near future.

VII. Acknowledgments

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References

[1] 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*, editered by F.Iida, R.Pfeifer, L.Steels, and Y.Kuniyoshi, New York, Springer, pp.130-145, 2004.

[2] Z.C.Chao, D.J.Bakkum, and S.M. Potter, “Shaping Embodied Neural Networks for Adaptive Goal-directed Behavior,” *PLoS Comput Biol*, Vol.4, No.3, e1000042, 2008.

[3] D.Xydas, D.J.Norcott, K.Warwick, B.J.Whalley, S.J.Nasuto, V.M.Becerra, M.W.Hammond, J.Downes, and S.Marshall, “Architecture for Neuronal Cell Control of a Mobile Robot, in *European Robotics Symposium 2008*, editered by H.Bruyninckx, L.Preucil, and M.Kulich, Springer, Vol.44, pp23-31, 2008.

[4] S.N.Kudoh, R.Nagai, K.Kiyosue, and T.Taguchi, “PKC and CaMKII Dependent Synaptic Potentiation in Cultured Cerebral Neurons,” *Brain Research*, Vol.915, No11 pp.79-87, 2001.

[5] K.Warwick, “Implications and Consequences of Robots with Biological Brains,” *Journal of Ethics and Information Technology*, Vol.12, No.3, pp.223-234, 2010.

[6] S.N.Kudoh and T.Taguchi, “Operation of Spatiotemporal Patterns Stored in Living Neuronal Networks Cultured on a Microelectrode Array,” *Advanced Computational Intelligence and Intelligent Informatics*, Vol.8, No2, pp.100-107, 2003.

[7] L.M.A.Bettencourt, G.J.Stephens, M.I.Ham, and G.W.Gross, “Functional Structure of Cortical Neuronal Networks Grown in Vitro,” *Phisical Review*, Vol.75, p.02915, 2007.

[8] S.N.Kudoh, I.Hayashi, and T.Taguchi, “Synaptic Potentiation Reorganized 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, Reutlingen, Germany, 2006.

[9] I.Hayashi, T.Taguchi, and S.N.Kudoh, “Learning and Memory in Living Neuronal Networks Connected to Moving Robot,” *Proc. of 8th International Symposium on advanced Intelligent Systems (ISIS2007)*, pp.79-81, Sokcho, Korea, 2007.

[10] S.N.Kudoh, C.Hosokawa, A.Kiyohara, T.Taguchi, and I.Hayashi, ”Biomodeling System - Interaction between Living Neuronal Network and Outer World,” *Journal of Robotics and Mechatronics*, Vol.19, No.5, pp.592-600, 2007.

[11] I.Hayashi, M.Kiyotoki, A.Kiyohara, M.Tokuda, and S.N.Kudoh, ”Acquisition of Logicality in Living Neuronal Networks and its Operation to Fuzzy Bio-Robot System,” *Proc. of 2010 IEEE International Conference on Fuzzy Systems (FUZZ-IEEE2010) in 2010 IEEE World Congress on Computational Intelligence (WCCI2010)*, pp.543-549, 2010.

[12] I.Hayashi, M.Kiyotoki, A.Kiyohara, M.Tokuda, and S.N.Kudoh, ”Fuzzy Bio-Interface: Indicating Logicality from Living Neuronal Network and Learning Control of Bio-Robot,” *Proc. of the International Joint Conference on Neural Networks (IJCNN2011)*, pp.2417-2423, 2011.

[13] B.Schweizer and A.Sklar, “Associative Functions and Statistical Triangle Inequalities,” *Publicationes Mathematicae Debrecen*, Vol.8, pp.169-186, 1961.

[14] I.Hayashi, E.Naito, and N.Wakami, “Proposal for Fuzzy Connectives with a Learning Function Using the Steepest Descent Method,” *Japanese Journal of Fuzzy Theory and Systems*, Vol.5, No.5, pp.705-717, 1993.