TEMPERATURE DEPENDENCY OF THE MAXIMUM CALCIUM CONDUCTANCE IN *Aplysia* NEURONS

Nam Gyu Hyun*, and Kwang Ho Hyun**

* Cheju National University, Jeju, KOREA
** Korea Science Academy, Busan, KOREA

nhyun@cheju.ac.kr

Abstract: We have measured the signals generated from big cells in the left rostral quarter abdominal ganglia of *Aplysia juliana*. When the temperature of the culture fluid containing the bursting cell was raised and lowered between 32°C and 10°C for about 40 minutes, firing patterns changed variously. Findings of this experiments showed that firing patterns of bursting cell changed into square-wave bursting pattern from parabolic bursting while temperature increased higher. Mean inter-spike intervals, mean duration time interval of inter-burst hyperpolarization etc. were analysed by using C^{++} programme, and relative error about experimental and theoretical values of these two were to be shortened within 10% using Mathematica programme. As a result we obtained a temperature dependency of the maximal calcium conductance and the maximal inactivation relaxation time constant of fast current.

Keywords: **Calcium conductance, Inactivation relaxation time constant, *Aplysia kurodai, Aplysia juliana*, Parabolic bursting, H-H model, Neuronal Model, Mathematica.**

Introduction

Aplysia californica has been used for the experiment to research the physiological function of the neurons since 1940, because it has large neurons of 50 – 500 μm diameter. Since then the positions of ganglia and the action potential patterns of individual neurons have been found. In particular cell biological approach on learning and memory has been mainly made[1]. But there is no habitat of *Aplysia californica* at the coastal sea of Jeju Korea, therefore instead of *Aplysia californica*, *Aplysia kurodai* and *Aplysia juliana* rich in Jeju have been used for the experiment.

Aplysia kurodai appeared to be identical to *Aplysia californica* in both anatomical and physiological properties of the nervous system. *Aplysia juliana* could be distinguished from *Aplysia californica* in certain morphological aspects of the nervous system[2].

This paper traces the change of firing pattern of *Aplysia juliana* depending on the temperature. The bursting cells are obtained in abdominal ganglia of *Aplysia juliana*. One bursting cell making regular spiking pattern for a long time at room temperature showed very various signal patterns such as regular beating, parabolic bursting, and square-well bursting at the temperature between 10°C and 32°C.

How the membrane potential varies with the flow of ionic currents can be simulated through the use of mathematical models, and we wanted to apply Nuronal Model suggested by Chay and Lee [3] to simulate these experimental results.

In this paper we examine the dependency of firing patterns in bursting cells at abdominal ganglia of *Aplysia juliana*, and simulate them by using Neuronal Model, and then we suggested the quantitative temperature dependency of maximal calcium conductance and the maximal inactivation relaxation time constant.

Materials and Methods

*Animals and D*issection

Many *Aplysia juliana caught* by women divers in Jeju Korea were raised and fed green lettuce in water tank of 17°C, and more than 60 were used for the experiments. These animals were dissected to observe how the signal patterns of the neurons of abdominal ganglia changed by the variation of temperature. The bursting cells keeping spiking condition for appro 300 seconds at the temperature between 20°C and 24°C were found to show so various signal patterns between 10°C and 32°C.

The process of dissection is as follows: firstly *Aplysia juliana* weighed 220 grams was injected with 110 *ml* of 0.38M $MgCl_2$ for anesthesia and abdominal ganglia were obtained by cutting the neighbouring neurofibril. Then these abdominal ganglia were soaked in L-15/ASW containing 8 mg/ml protease (type IX). The sheath of neurons was softened in the water bath circulator(JEIO-TECH, Model WBC-1510D) of 34°C for one hour. After being cleaned with artificial seawater, they were kept in the Low Temp. Incubator (HANBACK CO., Model HB-103MP) at 18°C for 10 hours. Finally they were removed on a petri dish (50mm×9mm), treated with sylgard and we pinned the ganglia very tightly. The peel was removed with fine scissors.

Eectrophysiology and Data Acquisition

We made a microelectrode out of a microcapillary (Glass Thin Wall, 1.0mm. TW100F-4, World Precision

ISSN: 1727-1983

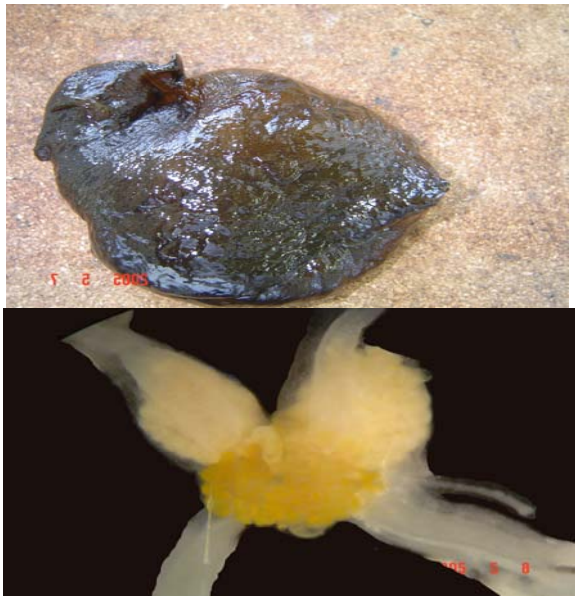

Figure 1: The upper picture is *Aplysia juliana* right before dissecting. The lower one is *Aplysia juliana's* abdominal ganglia taken in the experiment and was pinned on a sylgard plate. The electrode is inserted in the giant cell at the outskirts on the left in the lower picture).

Instruments, Inc.) using an electrode puller (Shutter Instrument Co., Model P-87) and filled it with *3M KCl*. The impedance of the microelectrode ranged from 10 to 20 $M\Omega$. The membrane potential of neurons in the intact abdominal ganglia of *Aplysia juliana* were measured by using Neuroprove Amplifier(A-M Systems, Inc,. Model 1600). These signals have been identified with digital Oscilloscope (Agilent, Model 54622A). A DAQ card (National Instruments, Model SCB-68) is connected between the amplifier and notebook computer (SAMSUNG, MODEL SEMS 830). Sixty files were made to be successively saved in the notebook at once, with one file being saved every minute through channel 1.

A petri dish with abdominal ganglia was put on two thermoelectric coolers (ACETEC CO., PART NO HMN 3840) and the temperature was regulated, while the current was being changed from 0 A to 1.5 A with DC Regulated Power Supply (between 0 V and 12 V). Within approx 30 to 40 minutes, the temperature of the culture fluid increased to 32 °C from 10 °C and decreased to 10°C from 32°C for the same time interval. Those experiments had been performed repeatedly for a definite time.

A K-type thermocouple was soaked in the culture fluid and neighboring nerve cells. Digital thermometer (TOHO, Model TRM-006) were connected with the K-type thermocouple by DAQ. The information obtained through the connection was saved simultaneously in the second column in each file through channel 2 with the information in the first column through channel 1. We measured the signals generated from big cells, as shown

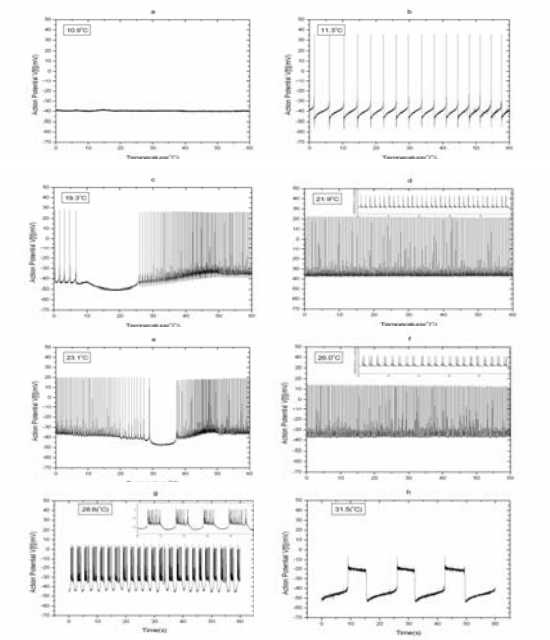

Figure 2: The change of firing patterns of *Aplysia juliana's* bursting cell according to temperature dependency is shown in these figures. (a) Silent state (10.8°C), (b) regular repeated beating (11.3°C), (c) starting point of bursting with long trains of action potentials (19.3°C), (d) middle point of bursting train (21.9°C), (e) end point of bursting train (23.1°C), (f) period doubling sequences (26.0°C), (g) bursting with short duration time of the inter-burst hyperpolarization (28.6°C), (h)relaxation oscillator (31.5°C), are shown from the top panel to the bottom one in succession.

in the lower picture of Figure 1, in the left caudal quarter abdominal ganglia of *Aplysia juliana* by raising and lowering the temperature between 10°C and 32°C. Data of temperature and action potentials were registered simultaneously at the rate of 3000 samples/s and 180,000 samples/channel by using an A/D converter with Labview.

Simulation

When the parameters used for Chay and Lee's Neuronal Model were properly combined, the parabolic bursting type should change into square-wave bursting. Out of 23 parameters used for Neuronal Model 21 parameters were given the proper solid numerical values. On the other hand the other two parameters, maximal calcium conductance and the maximal inactivation relaxation time constant of I_{Na} were adjusted to various numerical values for simulation.

Results

Firing patterns, such as relaxation oscillator, bursting within a short time of the inter-burst hyperpolarization, chaotic bursting, period doubling sequences, bursting with long trains of action potentials (about 300s) separated by silent periods (about 20s), regular repeated beating, silent states had been changed

ISSN: 1727-1983

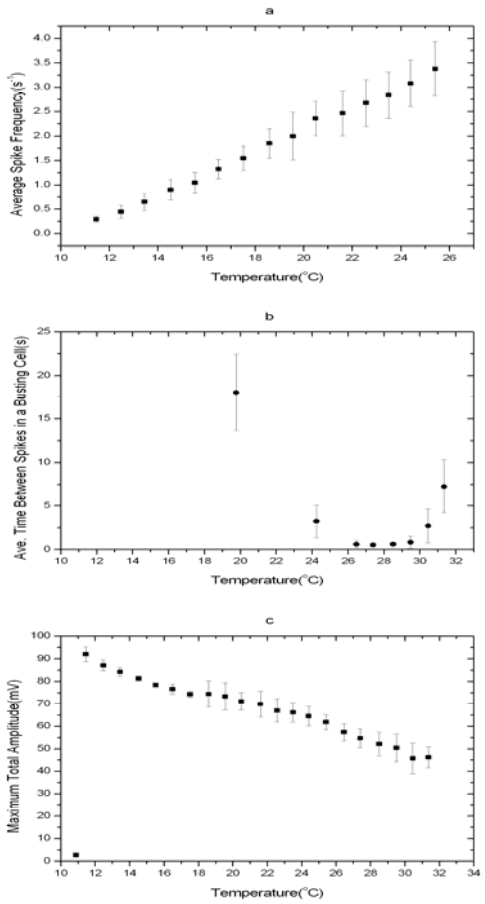

Figure 3: Analysis of 240 files using the C^{++} program. (a) Average spiking frequencies increase in straight line between 10°C and 32°C. (b) Bursting started at about 20°C and ended at about 24°C as shown in the middle of this figure; a small increase of the time interval of the inter-burst hyperpolarization above 26°C and big increase of the time interval above 30°C are also shown. (c) A total amplitude of action potentials decrease in a straight line according to temperature increase.

in order as the temperature was lowered as shown in Figure 2.

Average spiking frequencies, the time interval of the inter-burst hyperpolarization, total amplitude of action potentials, half-width of action potential, potential values at resting states, inter-spike intervals, had been analysed by using the C^{++} programme. The first three of them were analysed in Figure 3. Average spiking frequencies increased in a straight line between 10°C and 26°C, a small increase of the time interval of the inter-burst hyperpolarization above 26°C and big increase of time interval above 30°C was shown in b. The total amplitude of action potentials decreased in a straight line according to temperature increase.

Theoretical Model

All living cells were enclosed by a thin membrane and the excitable cell membrane could generate the electrical bursts. How the membrane potential varies with the flow of ionic currents can be simulated through the use of mathematical models. This model had evolved over a sequence of theoretical studies motivated by experimental data from the R-15 pacemaker neuron of *Aplysia*, perhaps the most widely studied bursting pacemaker. The Neuronal Model reviewed here is taken from Chay and Lee's paper[3].

In particular, the membrane can be thought of as a leaky capacitor. The membrane current i_m thus is the sum of the capacitive current and all ionic currents, viz.,

$$i_m = C_m \frac{dV}{dt} + \sum I_{ions}, \qquad (1)$$

where C_m is a more-or-less constant specific capacitance of the membrane (=1 μ F·cm^{-2}), V is the membrane potential, t is the time, and $\sum I_{ions}$ is the sum of all ionic currents, depending on the types of channels present in the membrane. Normally, i_m is zero, due to Kirchhoff's Law, which essentially restates the law of conservation for charge. That is, the capacitive and ionic currents are in balance.

$$C_m \frac{dV}{dt} = - \sum I_{ions}. \qquad (2)$$

The above expression forms the starting point for all mathematical models of membrane electrical activity following the Hodgkin-Huxley model theory. An important assumption is that all ionic currents flow independently of each other. In the Hodgkin-Huxley model, the ionic current carried by the y-type ions, I_y, may be expressed by a driving force multiplied by its conductance, where the driving force is the difference between the membrane potential and reversal potential, V_y. Thus I_y can be written as,

$$I_y = g_y (V_y - V). \qquad (3)$$

In eqn 3, the conductance g_y is proportional to the fraction of channels open at time t, y:

$$g_y = \bar{g}_y y, \qquad (4)$$

Where $\bar{g}_y$ is the maximum conductance, and y is a dynamic variable that can be solved by the following first order differential equation:

$$\frac{dy}{dt} = \frac{y_\infty - y}{\tau_y}, \qquad (5)$$

ISSN: 1727-1983

Table 1: Parameter values of the Model

Parameter	Unit	Numerical Value
C_m	$\mu F \cdot cm^{-2}$	1
$\bar{g}_{Na}$	$\mu S \cdot cm^{-2}$	1000
V_{Na}	mV	80
V_m	mV	-12
S_m	mV	6.4
V_h	mV	-40
S_h	mV	-6
V_{Ca}	mV	140
V_d	mV	-50
S_d	mV	7
V_f	mV	-54
S_f	mV	-10
$\bar{\tau}_d$	ms	1
$\bar{\tau}_f$	s	60
$\bar{g}_K$	$\mu S \cdot cm^{-2}$	330
V_K	mV	-80
V_n	mV	15
S_n	mV	15
$\bar{\tau}_n$	ms	80
$\bar{g}_L$	$\mu S \cdot cm^{-2}$	18
V_L	mV	-71

here, y_∞ is y at its steady state value, and τ_y is the relaxation time constant.

The membrane of this model contains a channel that has a feature similar to the Hodgkin-Huxley Na^+ channel, in that it is activated very fast and inactivated quickly upon depolarisation. There is a "slow" Ca^{2+} channel that contains a voltage-activated d-gate and a slowly inactivating voltage-dependent f-gate. There is also a Hodgkin-Huxley type K^+ channel whose gating is controlled by depolarisation(n-gate). In addition to these channels, the membrane contains a leak current of unknown nature.

Thus, in this model I_{ions} in eqn 2 can be expressed by a linear sum of four currents:

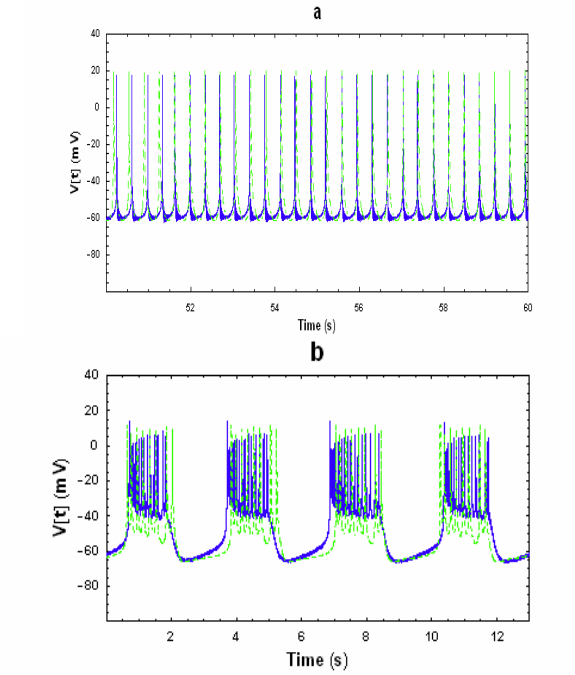

Figure 4: Comparison of the experimental results with simulation results, (a) beating at 24.3°C, (b) bursting at 29.8°C are shown in these figures. The solid lines represent the experimental results, and the dotted ones the simulation.

$$I_{ions} = \bar{g}_{Na} m_\infty h (V - V_{Na}) + \bar{g}_{Ca} df (V - V_{Ca}) + \bar{g}_K n (V - V_K) + \bar{g}_L (V - V_L), \quad (6)$$

where $\bar{g}_{Na}$, $\bar{g}_{Ca}$, $\bar{g}_K$, and $\bar{g}_L$ are, respectively, the maximal conductance of a voltage-gated Na^+, Ca^{2+}, channels, a voltage-activated K^+ channel, and a leak conductance; the V_{Na}, V_{Ca}, V_K, and V_L are the reversal potentials for the respective currents. The probabilities of openings, h, d, f, and n are dynamic variables that require the relaxation time constants $\bar{\tau}_h$, $\bar{\tau}_d$, $\bar{\tau}_f$, and $\bar{\tau}_n$, respectively. Since the opening of the m-gate takes place so fast, m is assumed by its steady state value m_∞.

In this model, the y_∞-curve with respect to V takes a sigmoidal shape and is described by a Boltzmann form,

$$y_\infty = \frac{1}{1 + e^{\frac{V_y - V}{S_y}}}. \quad (7)$$

That requires two parameters, the half-maximal potential V_y and the slope at the half maximal potential.

ISSN: 1727-1983

Table 2. Comparison of the experimental data with the simulated one of average spiking frequencies and average time interval between burstings.

Beating			Bursting		
Temp. (°C)	Ave. spike. Frequency (s^{-1})		Temp. (°C)	Ave. Time int. Between burst. (s)	
	Exp.	Simul.		Exp.	Simul.
11.75	0.405	0.390	25.65	0.388	0.356
12.07	0.254	0.251	26.12	0.366	0.362
12.77	0.692	0.691	26.27	0.368	0.333
14.19	0.518	0.520	26.85	0.387	0.354
15.4	1.295	1.324	27.60	0.335	0.335
17.06	1.524	1.538	28.06	0.457	0.477
17.47	1.680	1.663	28.67	1.22	1.125
17.76	1.069	1.050	29.52	2.395	2.51
19.58	2.123	2.109	29.75	1.939	1.823
19.69	1.953	1.934	30.77	6.024	5.701
21.53	3.125	3.164	31.16	7.854	8.170
22.90	3.215	3.164	31.51	10.56	11.378
24.13	3.875	3.745			
24.20	3.584	3.584			
25.19	3.745	3.676			

S_y for $y = h, d, f$, and n respectively. τ_y has the following form,

$$\tau_y = \frac{\bar{\tau}_y}{e^{\frac{V_y - V}{2S_y}} + e^{\frac{V - V_y}{2S_y}}} \qquad (8)$$

for $y = h, d,$ and f respectively, but τ_n has the following different form,

$$\tau_n = \frac{\bar{\tau}_n}{1 + e^{\frac{V - V_n}{S_n}}}. \qquad (9)$$

The membrane of this model contains two types of inward currents, fast and slow. A channel that is responsible for the fast inward current contains a quickly activating m-gate and an inactivation gate(h-gate) that closes rather fast upon depolarisation. A channel that is responsible for the slow inward current (*i.e.*, a slow Ca^{2+} channel) also contains an activation gate and closes very slowly upon depolarisation. In addition to these channels, the membrane contains a K^+ channel that is activated by voltage (*i.e.*, it contains the n-gate). All other currents (*e.g.*, the background $Na^+, Ca^{2+},$ or Cl^- currents, electrogenic pumps, and a Na^+ / Ca^{2+} exchanger) are included as a leak current in this model.

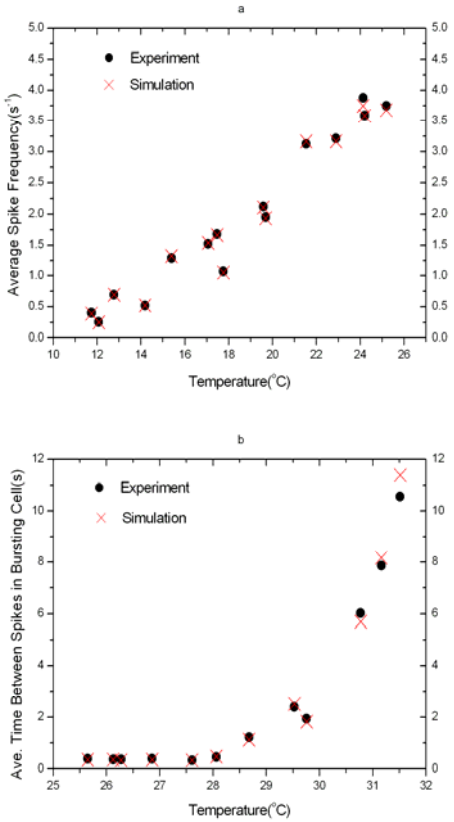

Figure 5: Twenty-seven comparisons of the experimental data with the simulated one at good data chosen between 10°C and 32°C are shown in these figures. (a) Good data files chosen between 10°C and 26°C, and per file, the relative error between the experimental value of average spiking frequencies and the simulated results are found to be below 5 per cent. (b) 12 good data files chosen between 26°C and 32°C, and per file, the relative error between the experimental values of the average time interval between bursting and the simulated results are found to be below 10 per cent. × in these figures means the experimental values, and • the simulated ones .

If we determine only two parameters (other parameters are fixed) of 23 parameters in the Neuronal Model appropriately, we can simulate the similar patterns of action potentials attained by the experiment. The values of the parameters present in this model can be found in Table 1.

We chose approximately 27 good data between 10°C and 32°C. At less than 25°C, the values of parameters are meaningful, when the relative error of experimental values of average spiking frequency showed less than 5 %. At more than 25°C, when in each file the relative error of experimental values and theoretical values of the time interval of the inter-burst hyperpolarization values became less than 10 %, as an example, we showed two of them in Figure 4. Then those two parameter values were chosen and the results were shown in Table 2 and Figure 5.

Based on these simulation results, we had the change of the maximal calcium conductance and maximal

ISSN: 1727-1983

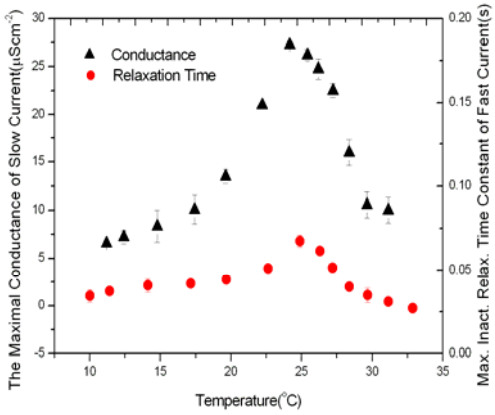

Figure 6: Based on the simulated results of Figure 5, changes in the maximal calcium conductance and maximal inactivation relaxation time with a fast current constant on temperature dependency are shown in this figure. The maximum value of two parameters appears at about 25°C, and the slope is higher at high temperatures rather than at low.

inactivation relaxation time constant of fast current on temperature dependency and these are shown in Figure 6.

Discussion

This paper traced the following fact: an increase in temperature caused a regular and reproducible increase in the frequency of pacemaker potentials in most *Aplysia* neurons[4]. In addition we observed that one bursting cell of abdominal ganglioa of *Aplysia juliana* produced various firing patterns on temperature dependency. In order to find the mechanism behind these findings, computer simulation using Mathematica had been utilized, and parameter values of Neuronal Model were to be determined. Out of 23 parameters, 21 parameters were made constant, and the other two parameters such as maximal calcium conductance and maximal inactivation relaxation time constant of fast current were made variables. As a result 27 various experimrntal firing patterns between 10°C and 32°C were simulated within 10 per cent relative errors.

The figure on the temperature dependency of the two parameters was produced in Figure 6.. Two parameter values continued to slowly increase from 10°C on temperature dependency. And at the temperature of 25°C values had maximum and thereafter parameter values decreased rapidly to 32°C.

The experimental proof as to whether maximal calcium conductance may vary on temperature dependency would be required. Hakozaki etc. proved that GTP-binding protein can be activated thermally using *Aplysia*[5]. Chay formulated a mathematical model for bursting neurons, which included a voltage-independent calcium channel[6]. Thus finally future experimental proof for these findings would be required.

Conclusions

We traced that firing patterns from bursting cells of *Apysia juliana* changed variously between 10°C and 32°C. In addition after these patterns were simulated using a neuronal model, we found that maximal calcium conductance and maximal inactivation relaxation time constant of fast current increased very similarly to 25°C on temperature, and then they decreased.

References

[1] KANDEL E. (1976): 'Cellular Basis of Behavior', (Freeman, W. H., New York), pp. 1-662

[2] LIM C.-S., CHUNG D. Y. ,and KAANG B.-K. (1997): 'Partial Anatomical and Physiological Characterization and Dissociated Cell Culture of the Nervous System of the Marine Mollusc *Aplysia kurodai*', *Mol. Cells.*, **7**(3), pp. 399-407

[3] CHAY T. R., LEE Y. S. (1990): 'Bursting, Beating, and Chaos by Two Functionally Distinct Inward Current in Activations in Excitable Cells', A*nn, N. Y. Acad. Sci.*, **591**, pp. 328-350

[4] CARPENTER D. O. (1967): 'Temperature Effects on Pacemaker Generation, Membrane Potential, and Critical Firing Threshold in *Aplysia* Neurons', *The Journal of General Physiology*, **50**, pp. 1469-1484

[5] HAKOZAKI S., MATSUMOTO M., AND SASAKI K. (1989): 'Temperature Sensitive Activation of G-Protein Regulating the Resting Membrane Conductance of *Aplysia* Neurons', *Japanese Journal of Physiology*, **39**, pp. 115-130

[6] CHAY R. C. (1996): 'Modeling Slowley Bursting Neurons via Calcium Store and Voltage-Independent Calcium Current', *Neural Computation*, **8**, pp. 951-976

ISSN: 1727-1983