The Effect of Heat on the Spiking Patterns of the Cells in *Aplysia*

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Fruitful findings have been produced from five out of sixty cells which were obtained from each 63 individual *Aplisia* caught at the Jeju coast. Spiking patterns of three out of five cells, such as relaxation oscillator, bursting within a short time of the inter-burst interval, chaotic bursting, period doubling sequences, bursting with long trains of action potentials separated by short silent periods, regular repeated beating or elliptic bursting, and silent states had been changed in order as the temperature was lowered to 10°C from 32°C. In the intervals of every about 40 minutes repeated ups and downs of temperature produced similar firing patterns at the allowable temperature ranges. The other two cells showed difference from these. The amplitudes of the action potentials of the two cells will not be highly decreased in 24 hours. Average spike frequencies, the inter-burst interval, peak to peak spike amplitude of action potentials, minimum potential values are compared and analyzed by using the computer programme. The spike frequencies according to temperature show the distribution of bell type, with maximal spike frequencies at intermediate temperatures and minimal ones at either end. The most common pattern consist of high spike frequency during falling and low one during rising temperatures.

Key Words: *Aplysia kurodai*, *Aplysia juliana*, Parabolic bursting, Square-wave bursting, Temperature

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.

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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 kurodai* in certain morphological aspects of the nervous system.[2]

Human beings have survived in the natural conditions where temperature gap shows big difference. Neuronal function of homeostasis which is apt to be constant temperature enables mankind to adapt themselves to the circumstances. Considering Krogh's principle, we think that *Aplysia* may be appropriate in the level of cells to prove the neurobiophysical mechanism that makes human being's body temperature constant.

Murray researched changes of impulse frequency, resting potentials, and resistence of neurons of abdominal ganglia of *Aplysia* according to temperature change.[3] Carperter released the findings on temperature effects on the frequency of spontaneous discharge and on the membrane potential and critical firing threshold.[4] Fletcher and Ram showed that interburst interval of *Aplysia* R15 bursting pacemaker neuron decreased to about 30°C from room temperature, and burst duration, spike height, and number of spikes per burst

decreased all with increasing temperature.[5] However, these findings suggested only the basic facts appearing on the limited areas of temperature with analytic data. Hyun and Hyun compared the results of computer simulation with the experimental digital data with eight firing patterns of action potential of one neuron in the left rostral quarter abdominal ganglia of *Aplysia juliana* and Hyun etc. analysed the digital data of the temperature and action potential from abdominal ganglia of *Aplysia juliana* and *Aplysia kurodai*.[6]

Fruitful findings have been produced from five out of sixty three cells which were obtained from each individual *Aplisia juliana* and *Aplysia kurodai* caught at the Jeju coast (6 *Aplysia*s weighted 0 to 100 grams; 48 *Aplysia*s 101 to 400 grams; 9 *Aplysia*s over 401 grams). These were dissected from January to July, 2005. After these are dissected, abdominal ganglia were obtained and put in culture solution. Then while the temperature of culture fluid was raised and lowered, the action potential was changed, and the results on the action potential depending on temperature difference were measured. Data of electric signals from 63 *Aplysia*s were saved in Hard Disk. However, data of short measurement, those not displaying exquisite bursting signals, and those not changing bursting patterns depending on temperature were excluded. Out of these saved data three cells changed bursting patterns depending on temperature and was given periodic temperature change stress over twice at least. And two cells were measured over 1,000 minutes, though bursting patterns did not change according to temperature. This paper demonstrated the analysis on those findings: bursting patterns of three neurons were changed and those of the other two were not changed, but remarkable changes in spike frequency and spike amplitude appeared.

MATERIALS AND METHODS

1. Animals dissection

Many *Aplysia* 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 difference by the variation of temperature, and abdominal ganglia were obtained and dealt with protease and peeled. The process of dissection is as follows: firstly *Aplysia juliana* or *Aplysia kurodai* was injected with 0.38 M $MgCl_2$ 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 Temperature Incubator (HAN-BACK CO., Model HB-103MP) at 18°C for 10 hours. Finally they were removed on a petri dish (50 mm×9 mm), treated with sylgard and I pinned the ganglia very tightly. The peel was removed with fine scissors. Dissection related on this experiment and the materials on obtaining the data are described on Table 1. These four *Aplysia juliana* and one *Aplysia kurodai* weigh from 130 g to about 360 g, which are appropriate for the experiment. It took about 50 minutes for their protease treatment.

2. Data acquisition

The abdominal ganglia were put in the culture solution and the fluid temperature increased and decreased between 10°C

Table 1. Data on dissection and measurement.

Animals	*Aplysia juliana 1*	*Aplysia juliana 2*	*Aplysia juliana 3*	*Aplysia juliana 4*	*Aplysia kurodai 1*
Date of exp. (month/day)	05/03	05/07	05/17	05/19	07/16
Weight (g)	246	220	137	358	290
Time for protease treatment (min)	64	44	42	52	50
Time for measurement (min)	1,120 (180)	350 (60)	420 (120)	360 (120)	1,940 (300)

( ): time for measurement in constant temperature.

and 32°C. Both action potentials from one neuron of abdominal ganglia and temperature were measured, and the data were automatically saved on the computer. We made a microelectrode out of a microcapillary (Glass Thin Wall, 1.0 mm. TW100F-4, World Precision Instruments, Inc.) using an electrode puller (Shutter Instrument Co., Model P-87) and filled it with 3 M KCl. The impedance of the microelectrode ranged from 10 to 20 MΩ. 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. A petri dish with abdominal ganglia was put on two thermoelectric coolers (ACETEC CO., HM 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

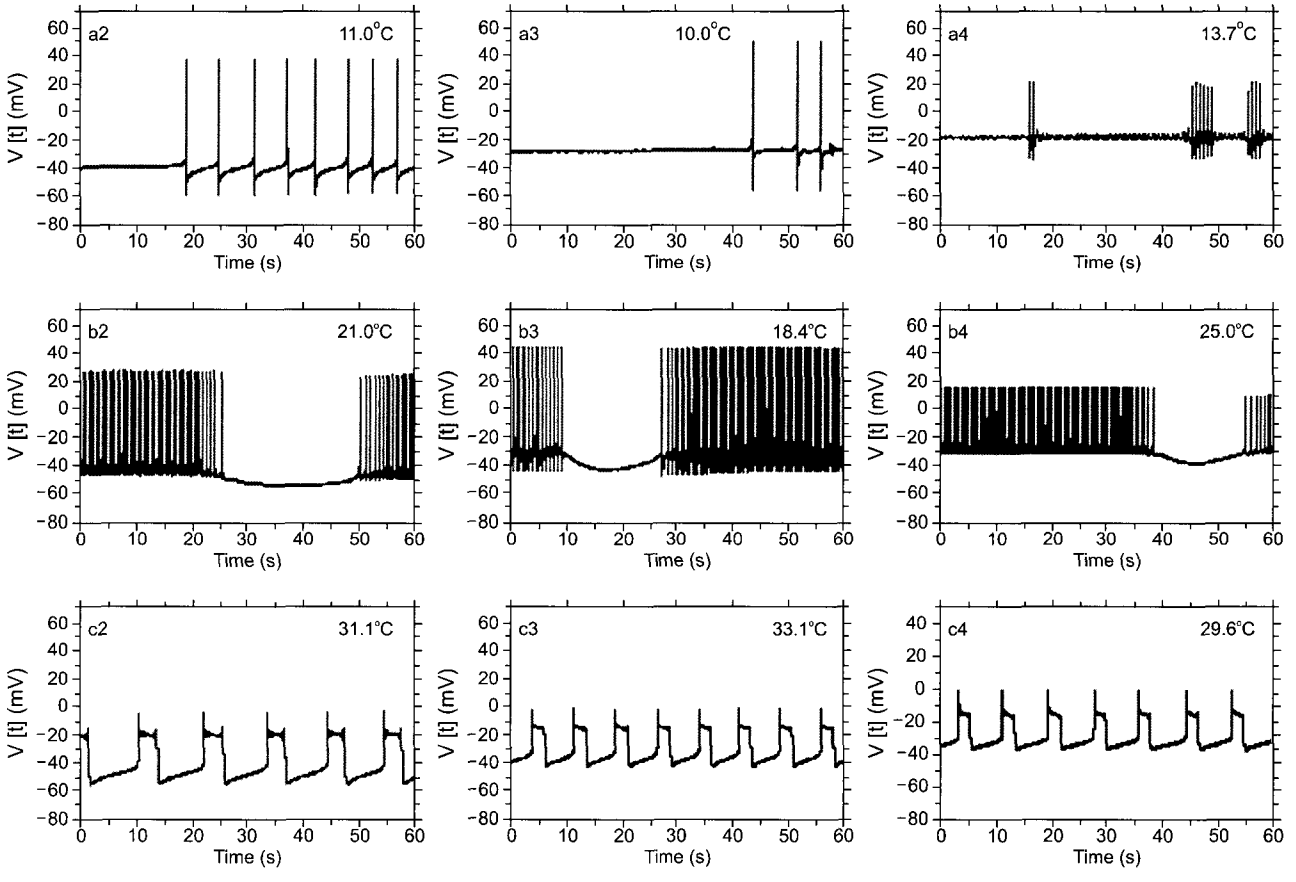

Fig. 1. Three panels on the left are the signals from *Aplysia juliana* 2: That of the upper left represents regular beating at 11.0°C; that of the middle parabolic bursting at 21.6°C; that of the lower left square-wave bursting at 31.1°C. Three panels in the middle are signals from *Aplysia juliana 3,* which is the same spike pattern of those from *Aplysia juliana* 2. Those are measured at the temperature of 10.0°C, 18.4°C, and 33.1°C from the top. Three panels on the right are those of *Aplysia juliana* 4: from the top elliptic bursting at 13.7°C; parabolic bursting at 25.0°C; and square-wave bursting at 29.6°C.

connection was saved simultaneously in the second column in each file through channel 2 with the information in the first column through channel 1. Data of temperature and action potentials were registered simultaneously at the rate of 3,000 samples/s and 180,000 samples/channel by using an A/D converter with Labview.

For the data, it took more than 1,000 minutes to measure the action potential for two neurons, and the other three neurons are consecutively measured from 350 to 420 minutes. For *Aplysia juliana 1*, both action potential and the temperature were measured for 1,120 minutes. Firstly for 60 minutes they were measured in constant temperature, and then about every 40 minute intervals they were repeatedly measured in the temperature between 10°C and 32°C. Between the intervals of middle and later part, the measurement was made in the constant temperature for 60 minutes respectively. After the experiment started, the temperature of *Aplysia juliana 2*, *3*, and *4* was constant for 60, 120, and 120 minutes respectively. And then they were measured in changing temperature between 10°C to 32°C. In the case of *Aplysia kurodai 1*, experiment continued for long hours. The temperature was made constant for first 120 minutes, mid 120, and later 60 minutes. The temperature of the other period was up and down repeatedly between 10°C to 37°C.

RESULTS AND DISCUSSION

The experimental data will be analysed into two categories. The one is the case that bursting patterns change according to temperature, and the other is the case that the bursting patterns do not change.

1. Case that bursting patterns change according to temperature

Aplysia juliana 2, *3*, and *4* are included in the case of bursting patterns change according to temperature. In *Aplysia*

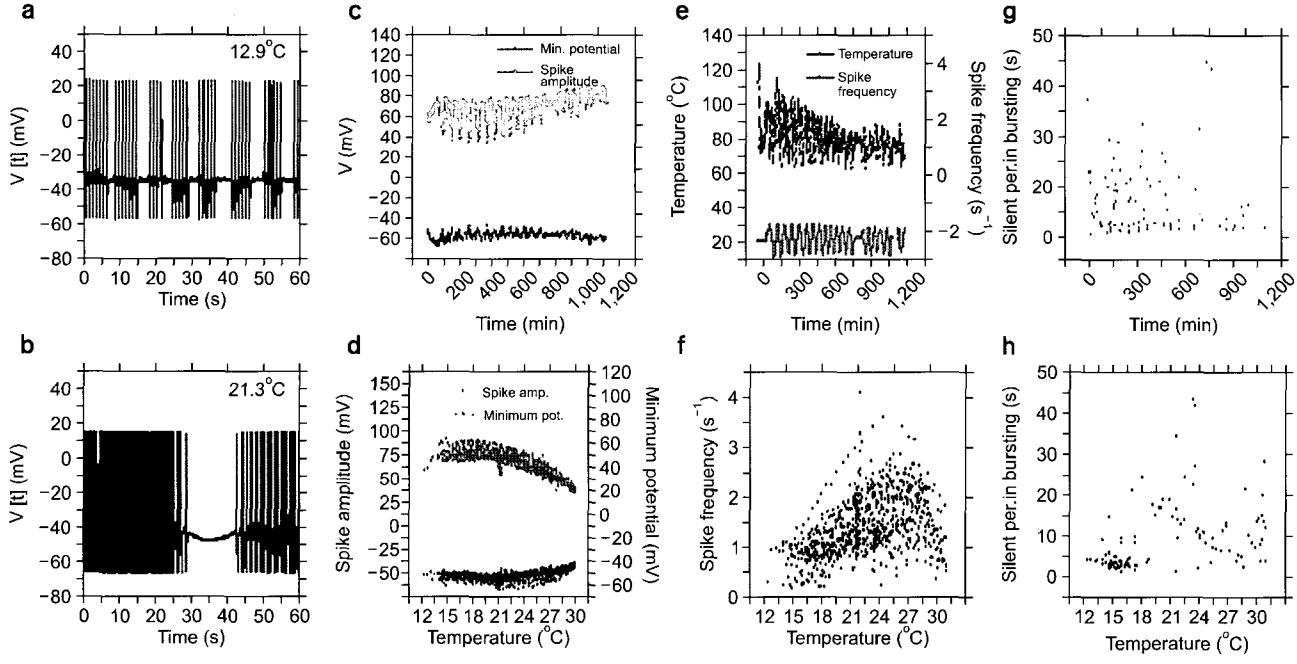

Fig. 2. Spike pattern and signal analysis of *Aplysia juliana 1*. Panel (a), (b) on the left show parabolic bursting signal at 12.9°C and 21.3°C. Panel (c) represents the change of spike amplitude (the above) and minimum potential (the below) according to time. Panel (d) is the identical content of panel (c), but shows the change according to the temperature. The next panel (e) represents spike frequency (the above) and the change of the temperature (the below) according to time. Panel (f) shows the difference of spike frequency according to the temperature. Panel (g) and (h) on the far right represent the interburst interval according to time and temperature respectively.

juliana 2, eight spike patterns were made during the temperature change from 10°C to 32°C.

Eight spike patterns were as follows: silent, regular beating, regular bursting (parabolic bursting) that spikes continue for a long time, period doubling sequences, period of four or eight or sixteen sequences, beating or bursting chaos, regular bursting (square-wave bursting), and square-wave bursting with short spike height and long interburst intervals. Three out of these eight spike patterns appeared on three panels of the left column of Fig. 1. The close analysis on these will be made in other paper. Another eight spike patterns in *Aplysia juliana 3* between 10°C to 32°C were similar to those of *Aplysia juliana 2*. However these patterns were not more distinctive than those of *Aplysia juliana 2*. Out of these the three important spike patterns are made in three panels in the middle of Fig. 1. *Aplysia juliana 4* did not start signal generation from silent state to regular beating around 10°C, but began from elliptic bursting type, which was different from *Aplysia juliana 2*, and *3*. The rest was similar to these two cases, and three pictures of Fig. 1 on the right explain this.

2. Case that bursting patterns do not change according to temperature

Aplysia juliana 1 and *Aplysia kurodai 1* are included in this case that bursting patterns do not change according to temperature. Their bursting patterns were irregular and they had no special regularity according to the temperature. During the experiment of 1,000 or 2,000 minutes, the amplitude of the

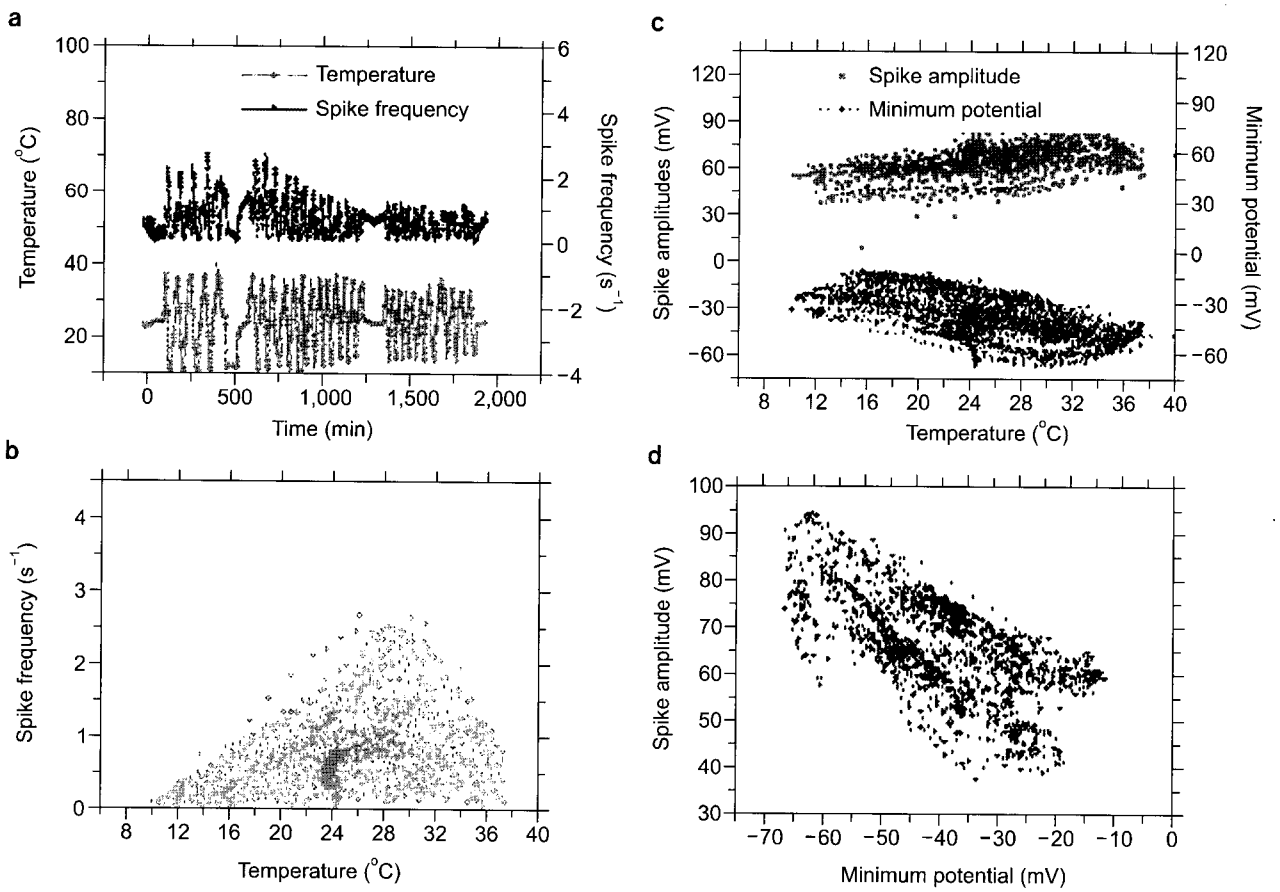

Fig. 3. Signal analysis of *Aplysia kurodai 1*. Panel (a) represents the difference of spike frequency and the temperature according to time. Panel (b) shows the change of spike frequency according to temperature. Maximum spike frequency appears at 28°C. Panel (c) on the upper right represents the change of peak to peak spike amplitude (above) and minimum potential (below) according to time. Panel (d) on the lower right show the interrelation of minimum potential (*x-axis*) versus spike frequency (*y-axis*).

bursting signals did not show big difference and the interburst interval were from 1 to 40 s continued. As we know from panel (a) of Fig. 2 on the left, the signal from the low temperature of *Aplysia juliana 1* is similar to bursting patterns from R15 pacemaker neuron of *Aplysia*. Like panel (b), when the temperature increased, the bursting pattern became parabolic bursting with long burst duration. The panel (c) and (d) of the second column from the left of Fig. 2 show both the change of peak to peak spike amplitude and minimum potential appearing from this neuron respectively according to time and temperature. As time passes, spike amplitude continues to increase. As the temperature increases, spike amplitude decreases, however minimum potential increases a little bit. Then the next two panel (e), and (f) explain the change of spike frequency according to the time and temperature. Panel (e) says that as time passes, the change of spike frequency decreases. And panel (f) explains that spike frequency is low around the low temperature 12°C and high temperature 30°C, but it has the maximum value around the temperature of 24°C. But spike frequencies according to temperature show the distribution of bell type. The two panel (g) and (h) in the far right elaborates the change of the value in interburst interval according to time and temperature change. Without any regularity of time and temperature, it changes irregularly.

Time to get the data in *Aplysia kurodai 1* is 1,940 minutes, which is about twice as long as that of *Aplysia juliana 1* (1,120 minutes). Nevertheless spike patterns from these two cells are very similar, because pannel (a) and (b) of Fig. 3 (*Aplysia kurodai 1*) and pannel (e) and (f) of of Fig. 2 (*Aplysia juliana 1*) show the very similar shape. Pannel (a) and (b) of Fig. 3 explain the change of spike frequency of *Aplysia kurodai 1* according to time and temperature.

However there are two differences in the spike patterns of these two cells. In the *Aplysia juliana 1* like panel (c) of Fig. 2, the size of peak to peak spike amplitude continued to increase from the start of experiment to the end, but in *Aplysia kurodai 1* this kind of distinction did not appear (a

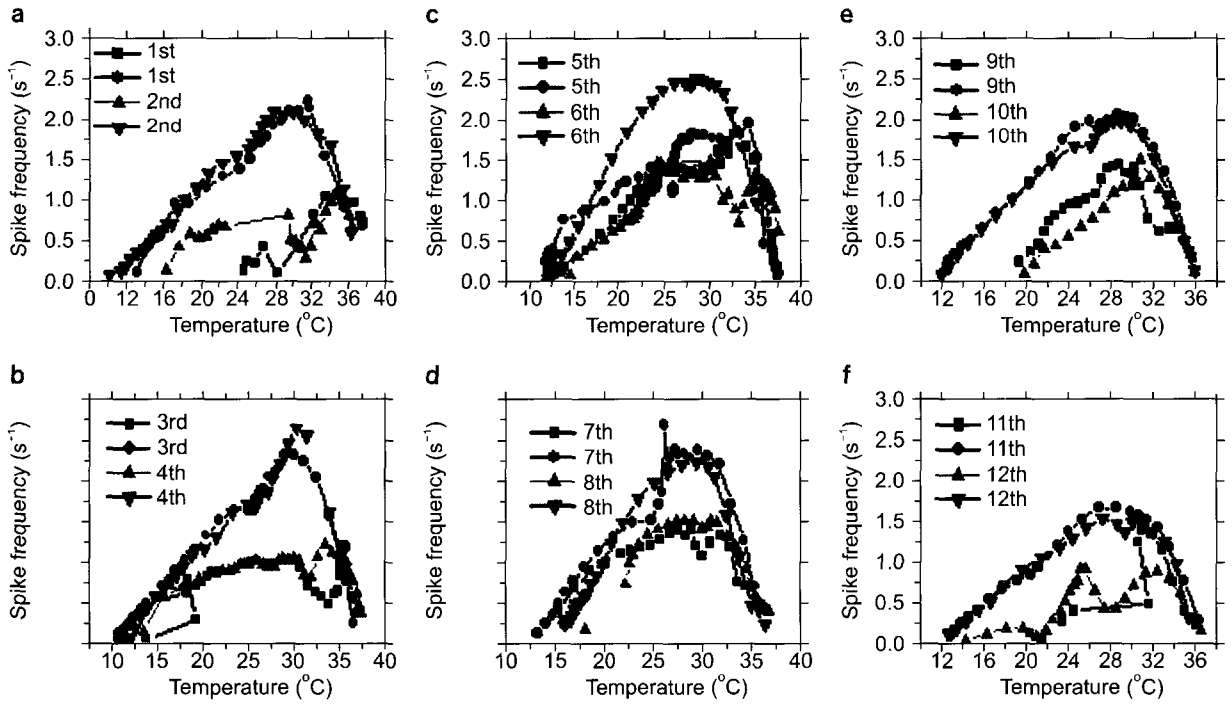

Fig. 4. Each panel of these figures show spike frequency of an abdominal ganglion of *Aplysia kurodai 1* as a function of temperature per twice periodic temperature change stress, but these panels represent detail signal analysis of panel (b) in Fig. 3. Lower curves in each panels, spike frequencies during rising temperature (■ and ▲); upper curves, during falling temperature (● and ▼). These shapes grade into humped types, with maximal spike frequencies at intermediate temperatures and minimal ones at either ends.

figure is omitted). When the temperature increases in *Aplysia juliana 1*, like panel (d) of Fig. 2, spike amplitudes decrease and minimum potential increase a little bit. But as we know from panel (c) of Fig. 3, in *Aplysia kurodai 1*, the opposite distinction appears. In Fig. 4 we showed detail signal analysis of panel (b) of Fig. 3. Lower curves in each panel represent spike frequencies during rising temperature and upper curves show these during falling temperatures. These shapes grade into humped types, with maximal spike frequencies at intermediate temperatures and minimal ones at either ends. The most common pattern consist of high spike frequency during falling and low one during rising temperatures. However as we know panel (d) of Fig. 3, when minimum potential (*x-axis*) increases, spike amplitude (*y-axis*) decreases in both cases (the figure of *Aplysia juliana 1* is omitted).

This paper demonstrated that the bursting patterns of the electric signal from cells of abdominal ganglia of *Aplysia juliana* changed regularly according to temperature. We think that the mechanism of these spike pattern transformation have to be proved on the point of theoretical physics. In addition even though the animals continue to receive stressful amount of heat stimulus with different temperature for more than 30 hours, some cells tend to keep spike patterns and intensity of signals to the end. If these characters of those cells are researched properly, we think those findings seem to be applied for the proper use.

CONCLUSION

This paper analysed the digital data of the temperature and action potential from abdominal ganglia of four *Aplysia juliana* and one *Aplysia kurodai*. In cells 2, 3, 4 of *Aplysia juliana*, the spike of regular beating or elliptic bursting started from silent states. In addition the spike pattern gets to change to square-wave bursting type through parabolic bursting type in the process of the temperature increase from 10°C to 32°C. When the temperature decreased, the opposite process appeared. Though long hours of stressful stimulus of temperature was given in *Aplysia juliana 1* and *Aplysia kurodai 1*, the important physical quantities including spike frequency and spike amplitude of action potential did not change significantly from the start of the experiment to the end. These shapes of the change of spike frequencies according to temperature, grade into humped types, with maximal spike frequencies at intermediate temperatures and minimal ones at either ends in *Aplysia juliana 1* and *Aplysia kurodai 1*. The most common pattern consist of high spike frequency during falling and low one during rising temperatures.

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군소 세포의 발화 형태에 미치는 열자극 효과

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현 남 규

제주 해안에서 잡은 60여 개의 군소를 대상으로 한 실험 데이터 중에서 5개의 세포에 대한 실험 결과를 분석해 보니 주목할 만한 결과들이 나왔다. 이들 5개의 세포들 중에 3개의 세포에서는 32°C에서 10°C 까지 온도를 내리는 동안에 이완된 진동 상태, 연속발화상태 사이의 과분극 기간이 짧은 버스팅, 혼돈 양상의 버스팅, 주기 배가 양상, 연속발화 기간은 길고 휴지 기간은 짧은 버스팅, 일정하게 반복되는 비팅 상태이거나 타원형 버스팅, 휴지상태가 차례차례로 나타났으며, 10°C에서 32°C까지 온도를 올리는 동안에는 그 반대 순서로 발화 형태가 변화하였다. 같은 온도 범위에서 80분 정도의 주기로 계속해서 열변화 자극을 줄 때마다 이러한 발화 형태 변화는 일정하게 나타났다. 그러나 나머지 두 세포의 경우에는 이와는 다른 발화 형태의 온도에 따른 변화를 보였다. 이들 경우에 이러한 열변화 자극을 24시간 이상 지속하였으나 발화 진폭은 크게 줄어들지 않았다. 그리고 평균발화 진동수, 버스팅 신호 사이의 평균 과분극상태 지속시간, 활동전위 진폭과 활동전위의 최저값 등을 C^{++}로 짠 프로그램을 실행시켜서 비교 분석 가능하였다. 온도에 따른 발화 진동수의 분포는 저온과 고온에서는 낮으나 중간 영역에서는 높은 종 모양의 형태를 보였는데, 온도를 내리는 동안에는 온도를 올리는 동안 보다 발화 진동수가 높았다.

중심단어: 참군소, 개군소, 포물선형 버스팅, 사각파 버스팅, 온도