Recording of bioelectric signal

Tamagawa Gakuen Upper Division SSH Resarch - Brain Science Grope -
H. Makiyama, R. Koh, K. Nakajima, Y. Fukuda, M. Tanimoto, R. Tanaka, T. Ota, Y. Takagi and A. Watari

[Motivation]

In this work, we investigated nerve structures and the operation mechanisms of the nervous system of a crayfish. It is very easy to investigate the the structure and operation mechanisms of this nervous system, because it is very simple in design. It is called the ladder-like nervous system[1].

American crayfish experiences simple reactions to outer stress. When it receives some external stimuli (stress) on the front part of its body, it escapes to the rear side, and, when it receives an external stimuli (stress) from behind, it escapes to front side[2]

In this work, we studied the structure of an American crayfish using dye technology. It is a useful method to see the ladder-like nerves. And we studied the operation mechanisms of the nervous system using electrical measurement technology.

[Experiment procedure]

(1)Anatomy of American Crayfish

Sample
American crayfish
Tools/materials used in this work
Anatomy plate, Surgical knife, Scissors for anatomy, Tweezers, Physiological saline
Procedure of anatomy
1. Anesthetizing
Sample (American crayfish) was put in a refrigerator for 30 minutes to stop moving. After 30 minutes , the crayfish was taken out of the refrigerator. And it was put on the anatomy plate with it's back facing up.

2. Anatomy
The upper half of the exoskeleton of the crayfish's cephalothoraxes were cut away using scissors. It is important that muscle tissue etc. adhered to the exoskeleton be removed carefully. The sample (American crayfish) was sunk in physiological saline to keep the internal organs alive

Fig. 1 Photographs of anatomy

(2)Observation of nervous system(structure)

Sample
American crayfish
Tools/materials used in this work
Anatomized American crayfish (shows in Fig. 1), methylene blue
Procedure of observation
1. Structure of the nervous system
After removing the internal organs and muscles, the nervous system appeared. It is called ladder-like nerves. As shown in Fig. 2, ladder-like nerves are composed of the main nerve along the center-line of the body and branch nerves extending outwards to the left and right side of the main nerve. The connection point of the main nerve and branch nerves is called a ganglion as shown in Fig 2.
American crayfish have a head ganglion, cephalothoraxes ganglion, 5 ganglions in the cephalothorax and 6 ganglions in the body. Each ganglion has two symmetric branch nerves as shown in Fig. 2. American crayfish have simple nervous systems that are eas

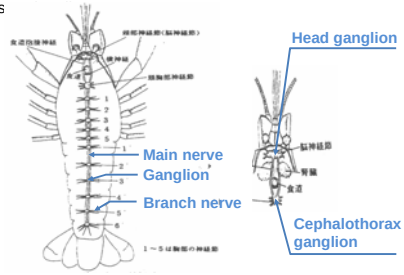

Fig. 2 Structure of ladder-like nerves

Therefore, we thought that it was a useful sample to investigate the nervous system using some optical and electrical methods.

2. Dyeing the nerve
Methylene blue (Solid chemical) shown in Fig.3 was dissolved with physiological saline. Methylene blue solution is often used for vital dyeing. In this work, It was used for dyeing the nerve. To dye the nerve, the anatomized American crayfish was sunk in methylene blue solution for 24 hours.

Fig. 3 Methylene blue

As shown in Fig. 4, the abdomen and scissors of anatomized American crayfish were dyed. After dyeing, the nerve was dyed with blue color.

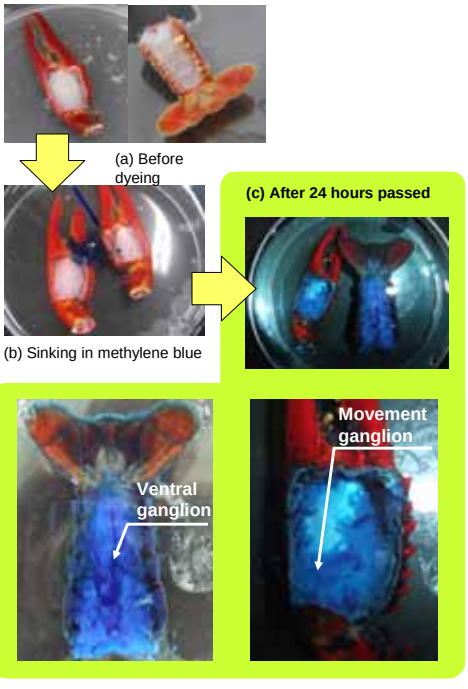

Fig. 4 dyeing process

Figure 4 shows that American crayfish has the main nerve and ganglions in the abdomen. And we can see the movement of the ganglion in the scissor.

(3)Investigation of the nervous activity by electric methods

Sample
Feeler of American crayfish

The electric potential signal that used the American crayfish's body was not able to be measured. In general, the measurement that uses the feeler is recommended. Therefore, we tried to measure the electric potential signal using the feeler of American crayfish.

Fig. 5 Feeler

Tools/materials used in this work
Oscilloscope, Amplifier, cable, Silver wire, Glass tube, Injection syringe, Schale, Manipulator, Physiological saline, Speaker, Microscope, Monitor(for microscope), Scissors, Tweezers made of plastic/wood, Rubber tube, Red clay
Procedure of measurement
1. Probe
To detect the electric potential signal from the feeler, the unique probe system shown in Fig. 6 was developed. The neuronal cell was drawn out to the inside of the glass tube by decreasing the in-tube pressure when the injection syringe is pulled, and the silver wire is connected to the neuronal cell.

The electric potential signal from the neuronal cell (nerve) is very small . Therefore, the silver wire that has very low resistivity was adopted. And it was insulated with the glass tube. It is important to decrease electric noise. The wire penetrated the rubber tube, and it connected to the amplifier.

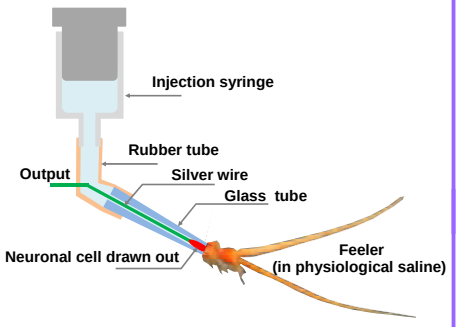

Fig. 6 Probe system

2. Circuit
To detect electrical potential signals of the neuronal cell (nerve) that reacted to the stimulation of the outside, we made a circuit shown in Fig. 7. The output signal that was transmitted by the silver wire was input to the amplifier. The amplifier enhanced the small electric potential signal of the neuronal cell (nerve). The amplified signal was measured by the oscilloscope. The amplifier and the oscilloscope were connected using a BNC(Bayonet Neill Concelman) cable to decrease electric noise. Using this measurement system, we demonstrated very low circuit noise as shown in Fig. 8. The detection of the electric potential signal of the neuronal cell (nerve) will be achieved using this measurement system.

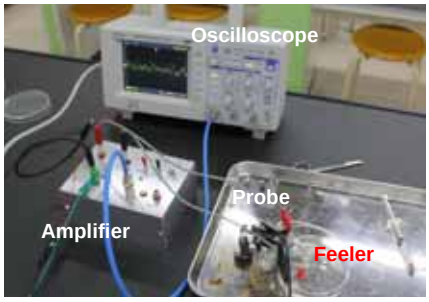

Fig.7 Circuit

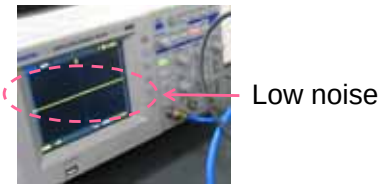

Fig. 8 Low noise circuit

[Summary]

We observed the nerve structure (ladder-like nerves) of an American crayfish using methylene blue dyeing method.
To detect electric potential signals of the nerves, we made a probe system and a low noise measurement circuit.
In this experiment, we studied [methods of processing sample] , [sample storage methods] and [noise decreasing techniques]. More improvements are currently being considered in the experimental methodology, and the detection of the electric potential signal of nerves.

[Reflection of the experiment / Improvements for the future]

- To measure the electric potential signal of the nerve, we will develop a technique that can better maintain the freshness of the sample.
- The electric potential signal of the nerve was very small. We will select an amplifier that can better measure the very small signal from the nerve.
- Conduct a comparative analysis of the nerve system of crayfish, using data collected from nervous system of other animals such as rats.

[Reference]

Publication in Japan
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渡辺 採朗 1996年
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2「ザリガニを主材とした甲殻類の実験」 大澤一爽
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