Galvanotaxis of the Plasmodium of *Physarum Polycephalum*

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Abstract. The traditional research method of the natural sciences chooses an element paying attention to the various elements in the natural world and analyzing their characteristics and components. To analyze the complicated structure of nature, one normally applies a highly precise device and the sophisticated expertise. This method will exclude other elements of the natural world, and will ignore mutual relations between elements that the network has. Such methods and results contribute to human profit immediately. On the other hand, by ignoring the function within the whole of the natural world, naturally we will face the environmental disruption threatening our survival. Thus modern technology resembles the person who is preoccupied with a specific thing in a forest, and loses his way. Because nature has a simpler aspect as a whole, it may not need the high quality technology for the understanding of nature as the whole. We have changed natural environments towards our profit for a long time. But, a protozoan (the lower animals) such as the myxomycetes let themselves adapt themselves to their environment by changing their lifestyle. Such a protozoan gives us valuable suggestions for our survival, and the new findings of a natural system provide a good opportunity to re-examine the scientific method. To understand nature as a whole, regardless of creatures and inanimate objects, it is necessary to understand how the systems of nature connect each other. Therefore, to obtain new findings on the mutual relations between environment and living creatures, in general, the ecosystem (or the behaviors of creatures) are investigated. The myxomycetes which have the time period of amoeba and a short life cycle, are considered best for observation of behaviors in environment.

In the plasmodium of *Physarum polycephalum*, we confirmed that galvanotaxis causes dilation of the tubular vein, the increment of resting potential, phase reversal of movement, and rapid flow of protoplasm streaming. In this paper, we show that the electric field strength can be used as an effective stimulus to motion control of a plasmodium on an agar-agar surface. (1) Galvanotaxic reinforcement: Our results show that the velocity of crawling increases in proportion to the DC electrical stimulus, up to a specific velocity. (2) Remaining galvanotaxis: A synthetic plasmodium composed of a experienced plasmodium which has been stimulated by the electric field strength and an inexperienced plasmodium which has not been stimulated, shows more rapid crawling than plasmodium which has not been stimulated. (3) Galvanotaxic application: In the experiment using a T-shaped path consisting of one path of feeble electric field strength and one with no electrical field, an experienced plasmodium, chooses almost always a path without the electrical field. On the other hand, the path chosen by an inexperienced plasmodium is always random. Our method has significant possibilities to find new findings for origin of memory and learning by a simple animal model, the plasmodium of *Physarum polycephalum*.

Keywords: *Physarum polycephalum*, galvanotaxis.

1 Introduction

To an organism, electrical stimulation produces various responses as well as the membrane potential [1]. In man, even if it is weak in strength, it activates to visual, auditory system. Therefore the electrical stimulation is applied to the diagnosis of eye disease such as pigmentary degeneration of the retina [2].

Galvanotaxis is well known in an amoebae and is used to escape from electric stimulation. From the investigation of Dictyostelium amoebae's electrotaxis, a recent paper suggests that electrotaxis and chemotaxis share similar signaling mechanisms. In this paper, researchers conclude that the pathways driving chemotaxis and electrotaxis intersect downstream of heterotrimeric G proteins to invoke cytoskeletal elements [3].

The presence of galvanotaxis, is well established when a constant current stimulation is given to the plasmodium of *Physarum polycephalum*.

While the plasmodium were showing avoidance behavior upon DC stimulation, in the tubular veins of the head and tail section, the number, diameter, and change of the protoplasmic streaming the inside were recorded.

The accompanied increase of the flowing quantity of the protoplasmic streaming, flow velocity, and the movement direction of the organism, and the expansion of the tubular veins of a leaf have also been confirmed [4,5,6].

In this study, we again used the plasmodium to investigate the changes in the velocity of crawling to the DC electrical stimulus frequency, and the remaining galvanotaxis of the synthetic plasmodium composed from a experienced plasmodium which has been stimulated by the electric field strength and an inexperienced plasmodium which has not been stimulated.

The purpose of this experiment is to investigate whether an intrinsic electric response level of organism can be changeable after it has received electrical stimulation. If the behavioral decision in an electrosensory system is confirmed experimentally, it is useful for new finding of the origin of memory and learning in the organism.

2 Method

1) Galvanotaxic reinforcement

A constant voltage power supply was used for experiments in the laboratory, or outdoors. Through a resistance of 200kΩ for prevention of overcurrent, 3V DC was applied to a plasmodium on the agar pool so that the current which flowed in an organism was 10μA. This is because it produced damage in the tubular veins when the current stimulation exceeds 1mA through a glass electrode [4]. The organisms whose weight was approx. 0.02 g, were raised on the agar nutrient medium.

In the experimental environment, an anode and a cathode were placed on both ends of the agar pool of size 1 x 1 cm. The surface of the agar pool was moistened with purified water (1 M Ω/100ml), and 3V DC was applied. A blob of oatmeal was placed over the cathode, whereas the organism was placed over the anode. 5 sets of the ager pool were used for experiment of the repeated stimulation. Ninety organisms were used.

When the organism arrives at a cathode, the organism is placed on the next agar pool with a pair of electrode. And 3V DC was applied again.

2) Remaining galvanotaxis

A new synthetic organism was composed from pairs of organisms. Both organisms whose weights were approx.0.01 g each, were used for experiment on the remaining galvanotaxis. One of them was an organism which had been stimulated one time at 3V DC. The other was an organism which had not been stimulated by an electric field.

3) The experiment using a T-shaped path

In the experiment using a T-shaped path having one path of feeble electric field strength and one of non-electrical field, the surface of the agar pool was moistened with purified water on the T-shaped path. Approx. 1μA DC current was applied on the right or the left path on T-shaped path. A blob of oatmeal was placed over both paths of T-shaped path, whereas the organism was placed in the front of T-shaped path, so that each organism can take a stimulated path or a non-stimulated path.

The experiment was conducted in two groups. One group contained thirty-one organisms which received electrical stimulation once at 3V DC. The other group is the same number of non stimulated organisms.

3 Results

1) Galvanotaxic reinforcement

Our results show that the plasmodium's velocity of crawling increases in proportion to the number of times it has been exposed to a DC electrical stimulus, up to a specific velocity as shown in Figure 1.

Figure 2 shows the membrane potential of typical organism. In the second stimulation of the organism, a higher resting potential following membrane potential is observed. Membrane potential differs at the recording site on the organism, and because of cyclic changeable potential. The increment of membrane potential, however, is recognized in general.

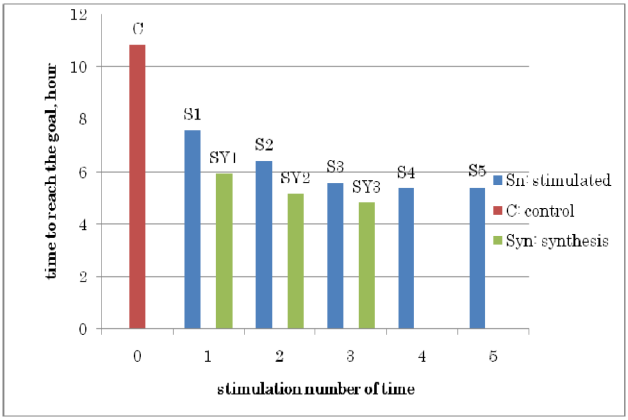

Fig. 1. The elapsed time of crawling from the onset of the stimulus. $p<0.05$ between control group C and one time stimulated group S1, S1 and S2, S2 and S3, S1 and synthesis group SY1, S2 and SY2, S3 and SY3. NS between S3 and S4, S4 and S5.

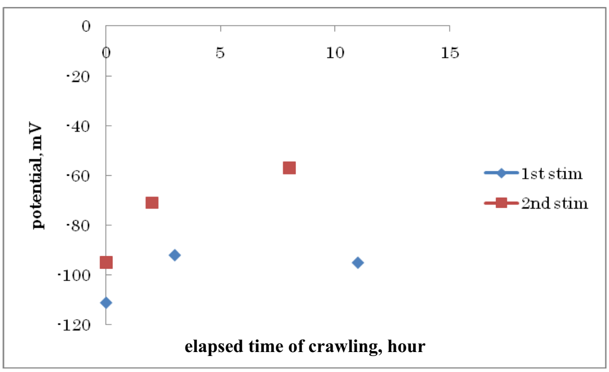

Fig. 2. Membrane potential on the head section of typical organism.

2) Remaining galvanotaxis

A synthetic plasmodium composed from an experienced plasmodium which has been stimulated by the electric field and an inexperienced plasmodium which has not been stimulated, shows more rapid crawling than a plasmodium which has not been stimulated.

3) The experiment using a T-shaped path

In the experiment using a T-shaped path having one path of feeble electric field strength and with no electrical field, an experienced plasmodium, chooses almost always a path of the nonelectrical field. On the other hand, the path chosen by an inexperienced plasmodium is always random. There is no relation between the position of the electrode and the choice of the path, each factor is independent respectively. (There is no significant difference.)

Table 1. The number of non stimulated organisms in the experiment using a T-shaped path at approx.1μA DC.

○ shows crawling away from the electrode.

● shows crawling towards the electrode.

Inexperienced. 71.0% Experienced 100% at approx.1μA DC. Both showed 100% correct rate at 4μA DC or more. On the Fisher's exact test of two-sided test at significance level 5%, 0.18 07＞2α＝0.1

	electrode position		
decision	left	right	total
○	12	10	22
●	3	6	9
total	15	16	31

4 Discussion

The reason for increment of the plasmodium's crawling velocity in proportion to the number of times is has been electrically stimulated, and the sensitivity of galvanotaxis of our composed plasmodium suggest that electrical stimulation gave to the organism a change in electrical features which reinforces galvanotaxis.

It is interesting that the organism continued still retains high electrical sensitivity even if electrical stimulation is stopped. The electric field strength may cause the change of electric features such as a conductivity, dielectric constant and time constant. As shown in previous research, furthermore, it causes mechanical changes such as the dilation of the tubular vein in the direction of the way to escape or making new network of the veins of the leaf by which the organism may minimize electrical damage [4].

The experiment using a T-shaped path suggests strongly that the experience of electrical stimulation changed the organism's behavior.

Our results can be useful to obtain new findings about basic knowledge of memory and learning for organism without brains.

References

[1] Kuroda, H.: Origin of the Membrane Potential in Plasmodial Droplets of *Physarum polycephalum*. J. Gen. Physiol., 637–655 (1981)

[2] Kato, S., Tanino, T., Saito, M.: The response of visual system evoked by alternating current. Medical and Biological Engineering 21(1), 47–51 (1983)

[3] Zhao, M., Jin, T., McCaig, C.D., Forrester, J.V., Devreotes, P.N.: Genetic analysis of the role of G protein coupled receptor signaling in electrotaxis. JCB 157(6), 921–928 (2002)

[4] Kato, S., Ebine, M.: Control of plasma streaming of *Physarum polycephalum* by Electric Stimulation. In: The Institute of Electronics, Information and Communication Engineers Proceedings of the 2008 IEICE Conference, vol. C-13, p. 54 (2008)
[5] Ebine, M., Kato, S.: Galvanotaxis by repeated electrical stimulation to the *Physarum* plasmodiums. In: Physiological Society of Japan Chubu 57 Conference 55 (2010)
[6] Ebine, M., Kato, S.: Animal model for human motion analysis. In: The 13th Japan-Korea Joint Symposium on Ergonomics (2011)