複合刺激に対する多細胞生物の応答
〜ボルボックスの走性のカップリング〜

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概要

本研究ではボルボックスが有する走光性と走電性に注目し、複合刺激に対し多細胞生物がどのように応答するかを調べた。まず光と電場をそれぞれ与える単一刺激の実験を行い、さらに光刺激と電気刺激を直角方向に組み合わせ、同時に刺激をあたえた。得られた結果は、

1. 単一刺激において、走電性の符号はかわらないが、光刺激の強度を大きくすると、走光性の符号は正から負に変わる
2. 複合刺激において、走光性と走電性のベクトルが合成される
3. 複合刺激において、単一刺激では見られなかった正の走電性が、負の走光性によって誘発される

Response of Multicellular Organism to Complex Stimulus
Coupling of photo-taxis and electro-taxis in Volvox

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Abstract

In order to reveal an integration mechanism of taxis, we have applied photo stimilus and electric stimilus perpendicularly at the same time to *Volvox* solution. The responce to photo and electric stimulus was analyzed by thier swimming directions when applied stimulus. In a single stimulus experiment, the probability distribution of direction of the swimming cells showed that photo-taxis changed its sign(positive to negative) as the intensity of light increased, whereas, electro-taxis does not change its sign(always negetive). In a comlex stimulus experiment, the probability distribution of direction of the swimming cells showed that large population of swimming cells moved in the direction which was the result of composition of two vectors(photo-taxis and electro-taxis). More surprisingly, we found that negative photo-taxis induced positive electro-taxis, and that this induced positive electro-taxis resulted in composition of two vectors(negative photo-taxis and positive electro-taxis).

1 Introduction

The behavior of microorganism has been described under the influence of a single kind of stimulation. But, normally in nature the conditions are as a rule more complex than this; the animal is affected by several sets of stimuli at once. "What is the behavior under such conditions?" if the animal is exposed to two types of stimuli a and b at the same time, "Which types of stimuli does the animal select to response?". Or will it, react in a new way, different from the usual reactions to either a or b. We would like to consider these responses of livning creatures from evolutionally primitive organisms. Volvox is a primitive multicellular organism evolved from single-cell organism, cryptomonas. The cells of *Volvox carteri algal* swim toward a light source or away from it. The direction of algal phototaxis is reversed by environmental factors. Halldal([9], [10]) found that the sign of phototaxis in *Platymonas* could be con-

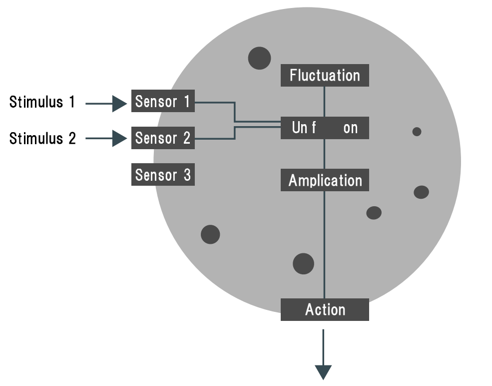

Figure1: Concept of internal state of living creatures.

trolled by changing the concentrations of magnesium, calcium and potasium ions in the medium. Further study of modification of the tactic sign by external ions, pH and chemicals was studied by Sakaguchi([5]).

The sign of phototaxis in *Volvox carteri* is also affected by another environmental facter, temperature of environment; positive at room temperature and negative at low temperature([4]). As multiple stimuli, when two factors in environment, ion concentration and temperature were changed, it was found that an increase in the potassium or hydrogen ion concentration raised the reversal temperature of the sign. Sakaguchi and Kozo([4]) concluded that the sign of phototaxis was determined by membrane polarization; on depolarization of the membrane the sign of phototaxis changes from positive to negative.

On the other hand, from celluler point of view, *Volvox carteri* is a spherical multicellular alga with many features that recommend it as a model for studying the process of cytodifferentiation([3]) and the early development of photoreception in eucaryotes. Individuals of this species contain only two distinct cell types, 16 large reproductive cells (gonidia) and from 2000 to 4000 somatic cells that cannot divide. The somatic cells are arranged in a single layer at the surface of the transparent sphere, whereas the 16 gonidia are located below the surface, where they have no direct contact with the external medium. All somatic cells are flagellated and possess eyes, and they are responsible for guiding the colony to places of light conditions that are optimal for photosynthetic growth([6]).

The orientation of the individual somatic cells within the spheroid, combined with the three-dimensional pattern in which their flagella beat, cause the spheroid to rotate in a counterclockwise direction. The two flagella of each cell beat synchronously and in an almost precisely parallel fashion. The flagella of all cells beat toward the posterior of the spheroid and slightly to the right, causing the spheroid to rotate to the left as it moves forward([11], [12]). Whether cells accelerate or decelerate in response to on and off stimuli depends on the light intensity, its illumination history and other environmental factors. Thus, in other words, colonial algae orient in light by a complex differential response of the cells at different sides of the colony and not by a differential response of the two flagella in an individual cell. Because algal colonies rotate more slowly than single-cell species, light-mediated signaling in an alga that exists in colonies is also expected to be slower than signaling in a single-celled alga.

Negative electro-taxis has been known for *Paramecium*([2]). organisms orient themselves with respect to the direction of current and move toward a negative electrode. *Volvox carteri* also have electro-taxis. In the present study, as a response to a single stimulus, we first examined the phototactic sign of *Volvox* as a function of light intensity. Secondly, electric field was applied, and the response of the swimming cells was studied. In order to answer the question, "Which types of stimuli deose the animal select to resonce?", photo stimilus and electric stimilus are applied perpendicularly at the same time to solution of *Volvox carteri*, and the response of the swimming cells was measured and discussed.

2 Materials and Methods

(*Plant material*)
Volvox carteri was cultured for 3 weeks under $12h$ of illumination of $2500[Lux]$ and $12h$ in darkness. Temperature is kept at $21[degree]$. The cell suspension in the log phase of growth was used as the experimental material. One liter of enriched Volvox medium contained 1mg $CaCO_3$ and $100g$ red soil. Cells were collected by $1000[Lux]$ white light and a concentrated part of the solution was taken out. This cell suspension were placed in incubator under darkness for 2 hours prior to experiment.

(*Measurement of photo-tactic and electric-tactic response*)
$3ml$ of *Volvox* solution was placed in a $30mm$ square acrylic pool. The photo stimulus was applied by white LED(Light Emitting Diode) in the acrylic pool in y direction. The electric stimulus was applied in x direction by aluminium plates which are placed in both sides of the acrylic pool. Thus, the photo stimulus and electric stimulus

can be applied perpendicular to each other in the square acrylic pool. Trajectory of swimming cells for 5 seconds is obtained from the moment when applied stimulus. Starting point is marked when applied stimulus and directions of swimming cells are obtained through vector which starts from starting point when applied stimulus to the end point which is the position of the swimming cell 5 seconds later. Applied external stimulus is described as follows;

1. The intensity of light was chosen for $78[Lux]$ and $152[Lux]$.
2. The intensity of electric field was chosen for $0.7[V/cm]$, $1.3[V/cm]$, $2.0[V/cm]$, and $2.7[V/cm]$.

In each experimental condition, response to photo stimulus and electric stimulus were represented in probabiliy distribution of swimming directions.

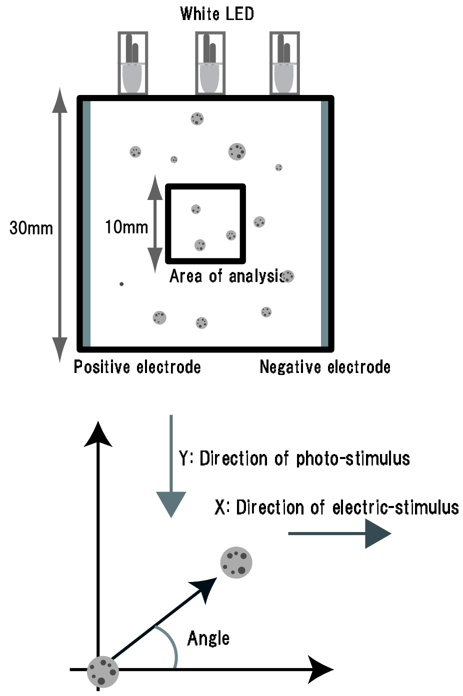

Figure2: Experimental set-up: *Volvox* solution was placed in $30mm$ square acrylic pool. The photo stimulus was applied by white LED in the acrylic pool in y direction. The electric stimulus was applied in x direction by aluminium plates on both sides. Thus, the photo stimulus and electric stimulus can be applied perpendicular to each other in the square acrylic pool.

3 Results and Discusion

3.1 Reponce to single stimulus

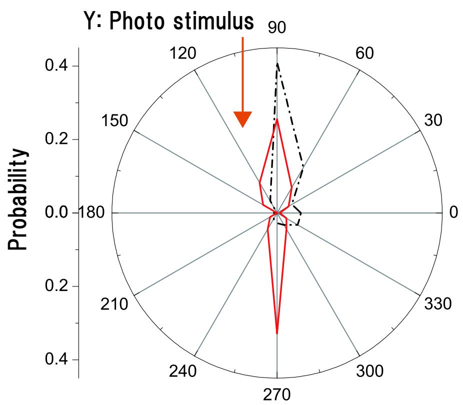

Figure3: The response to photo stimulus was represented in probabiliy distribution of swimming directions. Swimming direction of the cells was measured instantaneously when photo stimulus was applied to *volvox* solution. The applied light intensity was $78[Lux]$(dashed line) and $152[Lux]$(solid line).

The response to photo stimulus was represented in probabiliy distribution of swimming directions as shown in Fig.3. Swimming direction of the cells was measured instantaneously when photo stimulus was applied to *volvox* solution. The applied light intensity was $78[Lux]$(dashed line) and $152[Lux]$(solid line).

We discuss the photo-taxis of the swimming cells based on probability distribution of direction toward which the cell swims. When 78[Lux] light was applied to *volvox* solution, large population of the swimming cells moved toward the light, i.e., they showed positive photo-taxis. As intensity of light increased from 78[Lux] to 152[Lux], population was split into two distributions. About half of the swimming cells showed positive photo-taxis, and rest of the population showed negative photo-taxis. Furthermore, when the intensity of light increased to 244[Lux], most of the swimming cells showed negative photo-taxis. Thus, we found that *Volvox* changed the sign of photo-taxis as the intensity of light inceased.

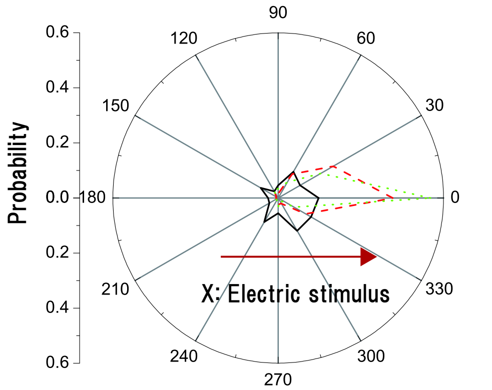

Figure4: The response to electcric stimulus was represented in probabiliy distribution of swimming directions. Swimming direction of the cells was measured instantaneously when photo stimulus was applied to *volvox* solution. The applied elecric field was 0.7[V/cm](solid line), 2.0[V/cm](dashed line), and 2.7[V/cm](dotted line).

The response to electcric stimulus was represented in probabiliy distribution of swimming directions as shown in Fig.4. When intensity of electric stimulus was 0.7[V/m], probability distibution of directions of the swimming cells slightly shift toward 0[$degree$]; small population of simming cells showed negetive electro-taxis. As intensity of electric-field increased to 2.7[V/cm], most of population showed negative electro-taxis.

When there is no stimulation in environment, cells swim toward all directions. Since intrinsic fluctuation is embodied in swiming cells, they swim toward randam directions. When applied electric stimulus, action of swimming cells seems to be based on ratio of two factors. One factor is random movement induced by the intrinsic fluctuation. The other factor is "decision of direction" to move toward the external stimulus, which is called taxis. As the strength of electric stimulus increases, "decision of direction" toward external stimulus increases more than intrinsic fluctuation, leading to a sharp distribution in probability distribution of the swimming cells. If we regard the swimming cell as a particle, this situation might coresspond to a situation in which Brownian particles are exposed to an external force field.

3.2 Response to complex stimulus

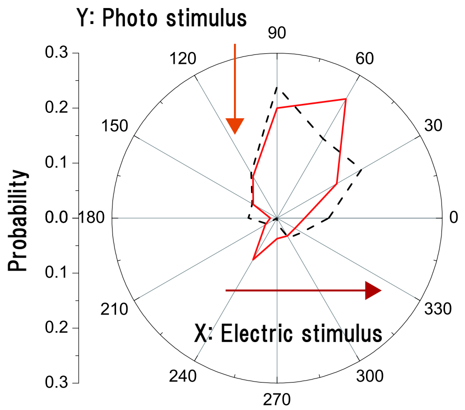

Figure5: The response to complex stimulus was represented in probabiliy distribution of swimming directions. Swimming direction was measured instantaneously when complex stimulus is applied to *volvox* solution(photo stimulus and electric stimulus were applied perpendiculary at the same time). The intensity of light was 78[Lux](dotted line) and 152[Lux](solid line). The elecric field(0.7[V/cm]) was applied for both measurements.

If the animal is exposed to two types of stimuli a and b at the same time, "Which types of stimuli does the animal select to response?". When electric stimulus and photo stimulus are applied perpendiculary, does the swimming cell choose one of the stimulus? or find a compromise between two stimuli by integrating information from two sense organ?

The response to complex stimulus was represented in probability distribution of swimming directions as shown in Fig.5. The elecric field(0.7[V/cm]) was applied for weak(78[Lux]) and strong(152[Lux]) photo stimulus. A large population of swiming cells moved toward light and some population slightly move toward the direction of 60[$degree$] when the light intensity was 78[Lux]. Slightly more than 20*percent* of population moved toward the direction of 60[$degree$] when the light intensity was 152[Lux]. Some population of the swimming cells moved toward the light, at the same time, they moved toward the negative electrode. They made a compromise between photo and electric stimulus.

Let us consider the direction of taxis as a vector as shown in Fig.7. In response to complex stimulus, some population of *Volvox* only shows photo-taxis, however, significant population of the cells swim toward the direction which is composition of two vector, namely, photo-taxis vector and electro-taxis vector.

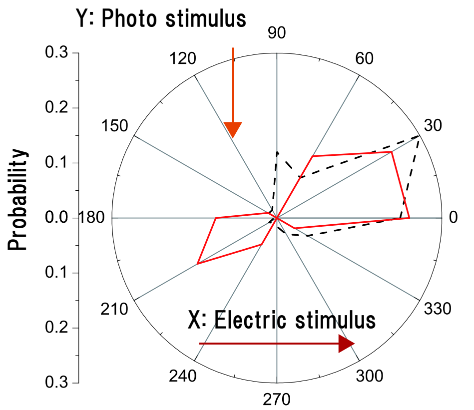

Figure6: The response to complex stimulus was represented in probabiliy distribution of swimming directions. Swimming direction was measured instantaneously when complex stimulus is applied to *volvox* solution(photo stimulus and electric stimulus were applied perpendiculary at the same time). The intensity of light was 78[Lux](dotted line) and 152[Lux](solid line). The electric field(2.7[V/cm]) was applied for both measurements.

The response to complex stimulus was represented in probability distribution of swimming directions as shown in Fig.6. The electric field(2.7[V/cm]) was applied for weak(78[Lux]) and strong(152[Lux]) photo stimulus. The results showed that a large population of swimming cells moved in the direction of composition of two vectors(photo-taxis and electro-taxis). Besides, about 20*percent* of the population move to the 30[*degree*] direction. This was considered as the result of composition of two vector when electro-taxis became stronger since the strength of electric field increased.

On the other hand, significant population of the cells moved to 210[*degree*] direction when the light intensity was 152[Lux]. In this light intensity, the result of response to photo stimulus in the single stimulus experiment showed that 22*percent* of the

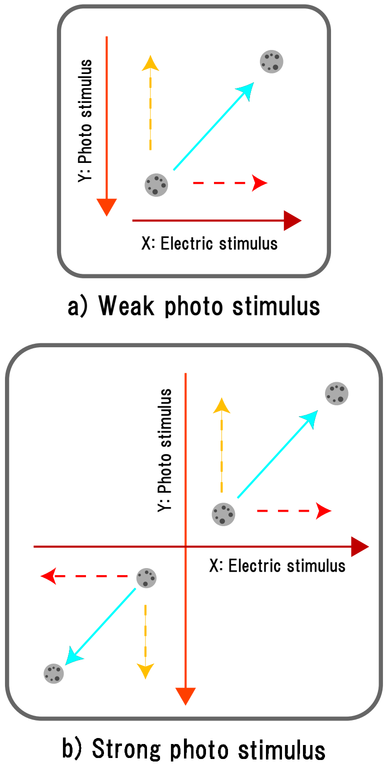

Figure7: Schimatic picture of taxis in response to photo and electric stimulus; Taxis of the swimming cells can be represented by vector, and the response of *Volvox* is represented as composition of two taxis vector(photo-taxis and electro-taxis). a) Weak photo stimulus: the intensity of light was 78[Lux]. b) Strong photo stimulus: the intensity of light was 152[Lux]. .

cells demonstrate negative photo-taxis. However, if negative photo-taxis and negative electro-taxis were composed, the swimming cells would move in the direction of 330[*degree*]. Thus, this result is somewhat controversy. We suggest that negative photo-taxis induce positive electro-taxis which is not demonstrated in single stimulus experiment, and that this induced positive electro-taxis resulted in composition of two vectors(negative photo-taxis and positive electric-taxis).

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