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Diversity in the Chemotactic Behavior of *Physarum* Plasmodium Induced by Bi-modal Stimuli

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The plasmodium of the true slime mold *Physarum polycephalum* is a unicellular, multinuclear giant amoeba that is attracting much attention in the field of bio-computing as a living computing substrate. To observe how the plasmodium of *Physarum polycephalum* responds to a contradictory situation, in which there is no single optimal solution, we applied bi-modal stimuli, consisting of a mixture of an attractant and a repellent, to plasmodia. The plasmodia showed diverse responses that could not be explained by a simple model of the stimulus-response system. We constructed a simulation model of the behavior that replicated the behavioral diversity with a simple combination of molecular apparatuses. In summary, we demonstrated the diversity of the behaviors of the plasmodium and how these behaviors may arise.

Keywords: Bio-computing; amoeba-based computing, *physarum* plasmodium, chemotaxis, behavior, decision making

1 INTRODUCTION

The plasmodium of the true slime mold *Physarum polycephalum* is a unicellular, multinuclear giant amoeba that is formed by the fusion of numerous

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uninucleate cells. In the late 20th century, the plasmodium was mainly studied as a model organism for cell biology because the large plasmodium cells enabled easy observation of cellular processes. Since Nakagaki et al. demonstrated that the plasmodium can solve a maze in 2000 [1], the organism has also attracted much attention in the field of computer science as a living computing substrate and is now used as a model organism for bio-computing. Following the maze-solving experiment, various experimental studies on *Physarum* bio-computing were performed, including optimizing networks [2,3], solving the traveling salesman problem [4], and duplicating real transportation networks [5,6] (for other representative experimental works, refer to [5,7,8]).

The utility of the plasmodium as a computing substrate has been extensively demonstrated. However, these previous studies are somewhat non-biological. For example, the studies in [1-6] were performed in closed spaces, and the optimization behavior of the plasmodium was used for computation. In contrast, we believe that an advantage of using a living computing substrate is that living organisms can adapt to open and unknown situations. In the previous studies, we thus performed an experiment to make the plasmodium learn a new situation [9], and experiments to observe the behavior of the plasmodium in an open space [10,11]. In this context, we attempted to give the plasmodium a contradictory situation, in which there was no single optimal solution. We gave the plasmodium bi-modal stimuli, consisting of a mixture of attractant and repellent. As a result, we observed diverse responses of the plasmodia to the bi-modal stimuli that could not be explained by a simple model of a stimulus-response system. Furthermore, we constructed a simulation model of the behavior and demonstrated that the behavioral diversity could be replicated by a simple combination of molecular apparatuses. In other words, we demonstrated the presence of diversity in *Physarum* logical operation and how it can be achieved.

2 MATERIALS AND METHODS

2.1 Plasmodium culture

All of the plasmodia used in this study were cultured using the method developed by Camp [12]. In a 30 cm $\times$ 20 cm plastic box, 9-cm glass Petri dishes were tightly arranged to minimize the space between them. Wet paper towels were placed on the dishes, and the space below the towels was filled with water to maintain the moisture in the box and on towel surface. Plasmodia were cultured on the paper towels, while the box was kept in the dark and the room temperature was maintained at 23°C. The plasmodia were fed daily

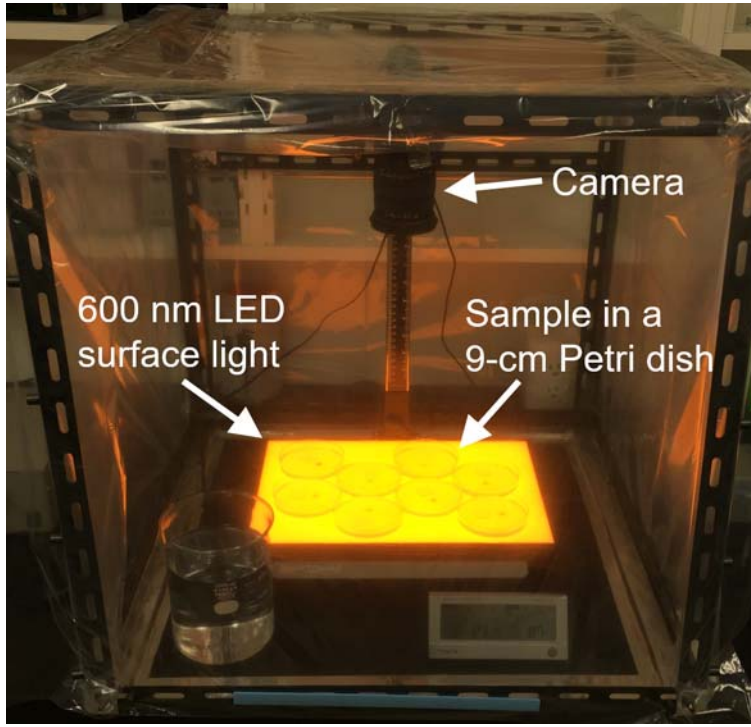

FIGURE 1
Photograph of the experimental setup.

with oatmeal flakes and within 24 h before the start of the experiments. The fresh locomotive fronts of the plasmodia were scraped, weighed, and used for the experiments.

2.2 Experimental setup

The experimental setup is shown in Figure 1. All experiments were performed in a hand-made cubic chamber assembled from 60-cm-long L-type steel angles covered in plastic sheeting to maintain the internal moisture. Before the experiment, water was sprayed throughout the inside of the chamber to supply moisture, and a 1-liter beaker of water was placed in the chamber to maintain the moisture levels. During the experiments, the chamber was kept closed, and the humidity in the chamber was maintained at greater than 80 %.

A digital camera (EOS Kiss X2, Canon, Japan) attached to a copy stand (King 731265, Asanuma & Co., Ltd., Japan) was placed in the center of the

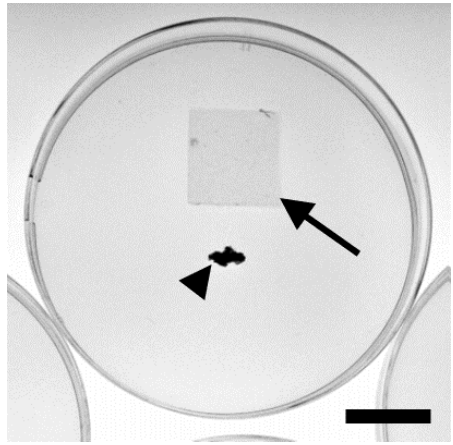

FIGURE 2
Photograph of the initial experimental condition. The arrow indicates a gel sheet of the bi-modal stimulant, and the arrowhead indicates the initial position of the plasmodium. (scale bar: 2 cm).

chamber. A surface light source consisting of 600-nm light emitting diodes (custom-made item, Aitec System Co., Ltd., Japan) was placed on the lower surface of the copy stand, and in the experiments, 8 samples on 1.5 % agar plates in 9-cm Petri dishes were placed on the surface light source. The light from the 600-nm light emitting diodes was used because this wavelength is outside the range that induces phototaxis in the plasmodium [13].

2.3 Bi-modal stimuli experiment

The mixture of attractant and repellent used as the bi-modal stimuli was prepared as follows. The attractant, which was 10 mg/ml crushed oatmeal (premium pure oatmeal, Nippon Food Manufacturer, Japan), and various concentrations of the repellent (10, 15, 20, 25, 50, 75, 100, 125, and 150 mM KCl; 163-03545, Wako Pure Chemical Industries, Japan), were mixed with 1.5% agar solution (010-08725, Wako Pure Chemical Industries, Japan). Thus, the strength of the attractant stimulus was constant and that of the repellent stimulus varied. Each solution was boiled and poured onto a plastic film (OHP film for PPC/laser printer, Kiso Chemical Corporation, Japan) to form a 1-mm-thick sheet.

The experimental field was a 1.5 % agar plate prepared in a 9-cm Petri dish. A 20×20 mm plastic film was placed on the agar plate 5 mm away from the center. The bi-modal stimulant, consisting of the agar sheet containing oatmeal and KCl was cut to the same size and placed on the plastic film, which acted as a barrier to prevent diffusion from the stimulant sheet into the agar plate.

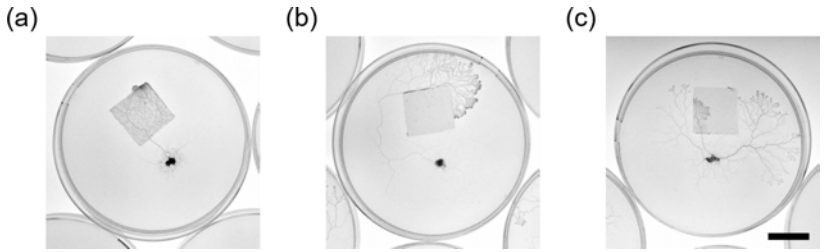

FIGURE 3
Photographs of examples of the responses of the plasmodia to the stimulant. (a) Complete attraction (10 mM KCl), (b) complete repulsion (100 mM KCl), and (c) intermediate behavior (50 mM KCl). (scale bar: 2 cm)

A portion of the cell body (30 mg) was scraped off each plasmodium that had been fed within 6 h and was used to inoculate the agar plate across the center 1 cm away from the stimulant sheet. Eight plasmodia were tested for each experimental condition, and scraped from the same plasmodium just before the experiment, except for the experiment with 50 mM KCl, which was performed with two sets of 8 samples (16 samples in total). Thus, all 8 samples were clones and they were almost identical in all biological aspects, such as gene expression level. The plasmodia in the experimental setup were photographed from above by the digital camera at 5 min intervals. Figure 2 shows the initial condition of the experiment.

The experiment was finished when the plasmodium reached the wall of the Petri dish. The ratio of the maximum area covered by the plasmodium during the experiment to the area of whole sheet, referred to as the coverage ratio, was used to evaluate the degree of the attraction or repulsion of the plasmodium. The ratio was measured by analyzing the images obtained in the experiment with image processing software, ImageJ [14].

3 EXPERIMENTAL RESULTS

Some examples of the experimental results are shown in Figure 3. The plasmodium was sometimes completely attracted to the stimulant (Figure 3a), sometimes completely repelled (Figure 3b), and showed intermediate behavior at other times (Figure 3c). The response was expected to vary according to the repellent concentration; however, the plasmodia showed diverse responses to the bi-modal stimuli, even at the same repellent concentration.

The coverage ratios for the experimental conditions are shown in Table 1, and the same data are compared visually in Figure 4. For 10 mM KCl repellent, all the samples were completely attracted to the stimulant and the

Conc. of KCl (mM)	Sample No. 1	2	3	4	5	6	7	8
10	1	1	1	1	1	1	1	1
15	0.713	1	1	1	1	1	1	1
20	0.023	0.116	0.153	0.383	1	1	1	1
25	0	0	0	0	0	0	0.086	0.845
50	0	0	0	0	0.028	0.234	0.522	0.899
50	0	0	0	0	0	0.005	0.494	0.81
75	0	0	0	0.071	0.281	0.343	0.347	0.511
100	0	0	0	0	0	0	0	0.495
125	0	0	0	0	0	0	0	0.576
150	0	0	0	0	0	0	0	0

TABLE 1
Coverage ratios for each experimental condition. The sample numbers are assigned in ascending order of coverage ratio. Coverage ratios of 1 are shown in red, those of 0 are shown in blue, and those > 1 and < 0 are shown in green.

coverage ratio was 1. For 15 mM KCl, 7 of the 8 samples were completely attracted to the stimulant, but 1 sample showed intermediate behavior, and was identified as an outlier. For 20 mM KCl, the responses of the plasmodia were split into 2 groups with a coverage ratio of 1 and a group with a coverage ratio of less than 0.4. For 25 mM KCl, an outlier appeared again, but with a higher coverage ratio. For intermediate KCl concentrations of 50 and 75 mM, the coverage ratio varied greatly; the responses of the plasmodia seemed

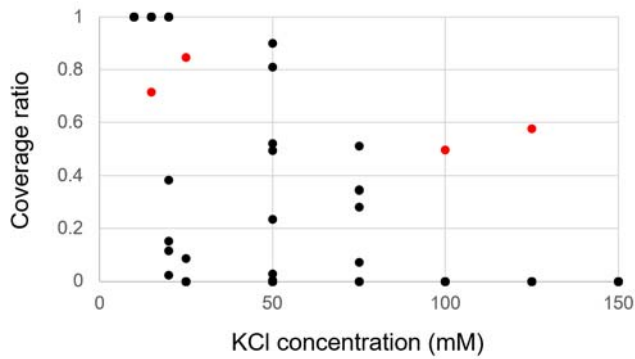

FIGURE 4
Scatter plot of the data in Table 1. The red points at each KCl concentration indicates one of the 8 samples that showed a different coverage ratio from the others and was classified as an outlier by Smirnov-Grubbs test (p ¡ 0.001).

chaotic and there seemed to be more than 2 patterns of behavioral choice. For 100 and 125 mM KCl, a single outlier with a higher coverage ratio appeared again, and finally for 150 mM KCl, all the samples were completely repelled the stimulant.

In summary, as the KCl concentration increased, the behavior of the plasmodium changed in the following order: complete attraction (10 mM KCl), binary choice (15, 20, and 25 mM KCl), chaotic (50 and 75 mM KCl), binary choice (100 and 125 mM KCl), and complete repulsion (150 mM KCl).

4 MODEL

To explain the experimental results and clarify the behavior mechanism, we constructed a simple signal transduction network model (Figure 5). We intended to construct a minimal model that replicated the experimental results. The molecular members of the model are as follows: receptors for the attractant (R_a) and repellent (R_r), signal transducer for the attractant signal (S_a) and repellent signal (S_r), and activating factor for the motor apparatus of cell motility (M). The receptors receive the input (stimulation) from the attractant (a) and repellent (r), and output signals are equivalent to the input ($R_a = a$, $R_r = r$). The transducers are activated by the signal from the receptors and inhibit each other. The pathway from the receptors to the transducer is simplified; for example, mediation by a secondary messenger is omitted. Furthermore, S_a activates M, and S_r inhibits M. The final concentration of M determines the behavior of the whole system; a high M value corresponds to the attraction of the plasmodium and a low value corresponds to the repul-

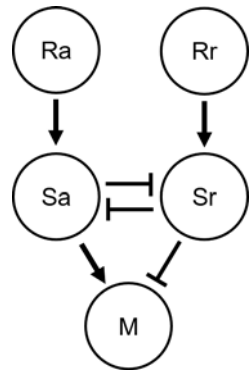

FIGURE 5
Schematic diagram of the model. The arrows indicate activation, and the T-shaped arrows indicate inhibition.

Item	Value	Description
v_{S_a}	1.0	Production (activation) rate for S_a
v_{S_r}	1.0	Production (activation) rate for S_r
v_M	1.0	Production (activation) rate for M
K_{R_a}	1.0	Dissociation constant for R_a activating S_a
K_{R_r}	1.0	Dissociation constant for R_r activating S_r
K_{S_a1}	0.1	Dissociation constant for S_a inhibiting S_r
K_{S_a2}	1.0	Dissociation constant for S_a activating M
K_{S_r1}	0.1	Dissociation constant for S_r inhibiting S_a
K_{S_r2}	1.0	Dissociation constant for S_r inhibiting M
D_{S_a}	1.0	Degradation (inactivation) rate for S_a
D_{S_r}	1.0	Degradation (inactivation) rate for S_r
D_M	1.0	Degradation (inactivation) rate for M

TABLE 2
Model parameter descriptions.

sion. The reactions of these members proceed via general Michaelis-Menten enzymatic reactions, and the receptors activate transducers in a dimeric way; thus, the dynamics of S_a, S_r, and M are given by following equations.

$$\frac{dS_a}{dt} = v_{S_a}\left(\frac{R_a^2}{K_{R_a}+R_a^2}\right)\left(1-\frac{S_r}{K_{S_r1}+S_r}\right) - D_{S_a}S_a \tag{1}$$

$$\frac{dS_r}{dt} = v_{S_r}\left(\frac{R_r^2}{K_{R_r}+R_r^2}\right)\left(1-\frac{S_a}{K_{S_a1}+S_a}\right) - D_{S_r}S_r \tag{2}$$

$$\frac{dM}{dt} = v_M\left(\frac{S_a}{K_{S_a2}+S_a}\right)\left(1-\frac{S_r}{K_{S_r2}+S_r}\right) - D_M M \tag{3}$$

Here, v_{S_a}, v_{S_r}, and v_M are reaction rate constants of each reaction, K_{R_a}, K_{R_r}, K_{S_a1}, K_{S_a2}, K_{S_r1}, and K_{S_r2} are the dissociation constants, and D_{S_a}, D_{S_r}, D_M are the degradation rate constants. The details of the parameters are described in Table 2.

In the simulation, attractant concentration a was set to mean 1.0 and variance 0.3. The average value of repellent r was varied and the values were r = 0.1, 0.3, 0.5, 0.75, 1.0, and 1.5. The variance of r was given by multiplying the mean by 0.3, and 1000 trials were run for each r value. The initial value of M was set to 0. Each trial was finished when M reached equilibrium.

5 MODEL SIMULATION RESULTS

The results of the simulation are shown in Figure 6. As r increased, the distribution of the concentration of M shifted to the lower side. In the conditions

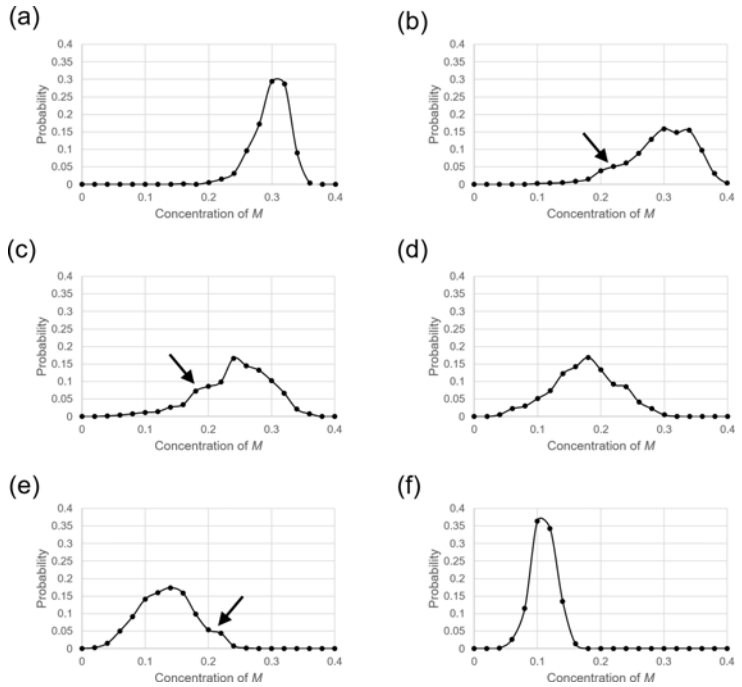

FIGURE 6
Probability distribution of the final concentration of M at equilibrium. Results from simulations with $r =$ (a) 0.1, (b) 0.3, (c) 0.5, (d) 0.75, (e) 1.0, and (f) 1.5. The arrows indicate the part of the distribution that shows weak bi-modality.

with the lowest and the highest r value, the distributions were uni-modal (Figures 6a and 6f). However, in some intermediate concentrations of M, weak bi-modality appeared in part of the distribution (Figures 6b, 6c, and 6e), and this corresponded to the outliers in the experimental results (Figure 4). When $r =$ 0.75, the distribution became broad (Figure 6d), corresponding to the experimental results with 50 and 75 mM KCl (Figure 4). The simulation results almost completely replicated the experimental result, if we assume that when the concentration of M is higher than 2.5, the whole system is completely attracted to the stimulant, and when the concentration of M is lower than 1.5, the system completely repels the stimulant, and when the concentration of M is between 1.5 and 2.5, an intermediate response appears.

6 DISCUSSION

To observe how the plasmodium responds to a contradictory situation, in which there is no single optimal solution, we applied bi-modal stimuli to

plasmodia. As a result, we found the diversity of the response of the plasmodium: in some case, the responsive behavior seemed to be bifurcated to 2 choices, and in the other case, it seemed to be chaotic. We constructed a model to explain the experimental results, and replicated the phenomenon.

Our experimental results indicate that, even in cell decision-making, there are always potential options that allow for the possibility of more than one behavioral choice. Usually, the presence of these options is attributed to fluctuations under the normal stimulus-response diagram. However, our experimental results demonstrated that cells have some degree of freedom in behavioral choice, though the limits of what options are possible may be determined by the set of molecular members in the cell and the genes in the genome. Therefore, *Physarum* bio-computing cannot be regarded as pure information processing processes, and should inevitably be regarded as, or modeled by computation that has physical properties [15-18]. Furthermore, we predict that the existence of the behavioral diversity is not limited to a particular function of a particular organism because our simple model with a small number of members nearly exactly replicated our experimental results, as Schumann pointed out that this is the case with biological systems at various levels [19].

In future work, we will continue our experimental and model studies. Owing to the small number of experimental samples, we have neither clarified the probability distribution of the plasmodial behavioral choice nor confirmed whether there is bifurcation and a chaotic state. Our model is sufficient to explain the experimental results, but the model is still a toy model and is not sophisticated in terms of systems biology and complex systems theory. However, we believe that our work will contribute to understanding the organism-specific mechanism of decision-making and logical structure of biological entities.

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