Time Delay Effect in a Living Coupled Oscillator System with the Plasmodium of *Physarum polycephalum*

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A living coupled oscillator system was constructed by a cell patterning method with a plasmodial slime mold, in which parameters such as coupling strength and distance between the oscillators can be systematically controlled. Rich oscillation phenomena between the two-coupled oscillators, namely, desynchronizing and antiphase/in-phase synchronization were observed according to these parameters. Both experimental and theoretical approaches showed that these phenomena are closely related to the time delay effect in interactions between the oscillators.

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A coupled nonlinear oscillator system with time delay is expected to demonstrate rich collective behavior such as multistable synchronization and amplitude death by the theoretical analysis [1–4]. Although the biological coupled oscillator system is a candidate for such a system, there is no experimental evidence directly indicating that the time delay effect exists in the biological systems. We proposed a living coupled oscillator system of the plasmodium of the slime mold, *Physarum polycephalum,* where coupling strength and delay time can be systematically controlled by a cell patterning method [5]. In this study, we show the time delay effect exists in this living system where various phase lock states, namely, antiphase and in-phase synchronization phenomena, were observed depending on two parameters that correspond to the coupling strength and the delay time.

The plasmodium of *Physarum* is an amoeboid multinucleated unicellular organism, which is an aggregate of protoplasm without any highly differentiated structure like a nervous system (Fig. 1a). Despite its simple structure, the plasmodium demonstrates sophisticated biological functions such as gathering/escaping behavior to/from attractants/repellents [6]. These functions can be realized through intrinsic nonlinear oscillatory phenomena in the plasmodium that are supposed to be generated by complicated mechanochemical reactions among intracellular chemicals such as ATP and Ca^{2+}, actin, etc. [7]. These oscillatory phenomena generate contraction/relaxation states in the plasmodium periodically, which derive a thickness oscillation in the plasmodium. The contraction/relaxation state also derives pressure difference among partial bodies of the plasmodium that are interconnected by tube structures (arrows in Fig. 1a). Accordingly, the pressure differences induce the shuttle streaming of the protoplasm inside the tube structures [8]. From the viewpoint of nonlinear dynamics, the plasmodium can be modeled as a coupled nonlinear oscillator system [9,10]. In this model, an oscillator can be defined for each partial body in the plasmodium and the coupling would be affected by the protoplasmic streaming. Therefore, by systematically controlling the dimension of the tube structure, it would be possible to observe a variety of oscillation phenomena.

We patterned the plasmodium by a microfabricated structure (Fig. 1b) based on the method we developed [5] and constructed the living coupled oscillator system which consisted of two plasmodial oscillators and a coupling part (Fig. 1c). The two oscillators were physically connected by the protoplasmic streaming in the tube structure of the coupling part. With this method, the coupling configuration, for example, a tube diameter (D) and a distance between oscillators, can be systematically controlled by the channel width (W) and length (L). In a narrow channel, a single tube structure was formed

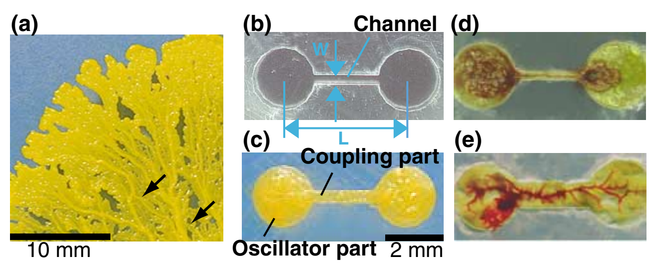

FIG. 1 (color). (a) *Physarum* plasmodium. (b) Microfabricated structure. (c) A living coupled oscillator system with the plasmodium patterned by the microfabricated structure that was made from an ultrathick photoresist resin (NANO™ SU-8 50, Microlithography Chemical Corp.) by a photolithography method. The structure, which consisted of a thick sheet (0.1 mm in thickness) and had a dumbbell shaped opening, was put on a 1.5% agar plate (without nutrient) to pattern the wet (agar) and dry (microfabricated structure) surfaces. The plasmodium spread only on the wet surface. The diameter of the oscillator parts was determined as 2 mm, which is comparable to half of the spatial wavelength of oscillation in the natural plasmodium, so that we can consider the plasmodium in the oscillator part as a single oscillator [5]. (d),(e) Tube structures formed in the channels of $L = 4$ mm. Only the tube structures were stained by a red dye solution (5mg/mℓ neutral red, 25 mM KCl, 1 mM NaCl, 10 mM morpholine propane sulfonic acid at pH 7.1). $W = 0.20$ mm in (d) and 0.80 mm in (e).

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(Fig. 1d) and the tube diameter was systematically controlled by the channel width. In a wide channel exceeding a certain critical width, a dendritic tube structure was formed (Fig. 1e) and the tube diameter was no longer systematically controlled by the channel width [5,11]. Therefore, we principally took into account the samples in which the single tube structures were formed.

We observed the thickness oscillation in this system. Various types of oscillatory phenomena, for example, antiphase and in-phase oscillation, etc., were observed depending on W and L. Figure 2 shows an example of antiphase oscillation, where alternating wave propagation from one plasmodial oscillator to another is shown. Figure 3 shows the time course of thickness in each oscillator. When L was small (4 mm) and W was relatively large (0.40–0.50 mm), two plasmodial oscillators were synchronized in antiphase (Figs. 2 and 3a). This phenomenon differs from that observed in a general coupled oscillator system such as a diffusion coupling system, where in-phase oscillation would be expected at the strong coupling. The reason for this result will be discussed later. When W became somewhat smaller (0.20–0.30 mm), the oscillators showed quasiperiodic antiphase oscillation (Fig. 3b). When W was much smaller (0.05–0.10 mm), no remarkable mutual entrainment between the oscillators was observed (Fig. 3c). When L was large (10 mm or more), the oscillators were synchronized in phase (Fig. 3d). Finally we obtained a phase diagram on the W-L plane, as shown in Fig. 4a, where there are three main features, namely, unentrained, antiphase, and in-phase oscillations (denoted as squares, circles, and triangles in the figure, respectively). In the shaded region, where W was large and the plasmodium in the channel formed dendritic tube structure (Fig. 1e) [5,11], the oscillations were complicated and the coherency between the two oscillators was small (denoted as asterisks). In the narrower channel (less than 0.50 mm), the tube structure was never formed [5], which means there was no coupling between the oscillators (denoted as crosses). The most interesting result is that the phase transitions from/to antiphase to/from in-phase were repeated as the parameters W and L increased.

This characteristic shown in this phase diagram was predicted by Schuster and Wagner [2] with the phase coupled equations as follows:

$$\begin{aligned} d\phi_1(t)/dt &= \omega_1 - K\sin[\phi_1(t) - \phi_2(t-\tau)], \\ d\phi_2(t)/dt &= \omega_2 - K\sin[\phi_2(t) - \phi_1(t-\tau)], \end{aligned} \tag{1}$$

which represent that the two oscillators with the phases ϕ_1, ϕ_2 and the intrinsic frequencies ω_1, ω_2 interact through the coupling strength K with the delay time τ.

In the plasmodial oscillator system, ϕ_1, ϕ_2 would correspond to the phases of the thickness oscillation in each oscillator part and ω_1, ω_2 would correspond to the frequency of oscillation observed in each oscillator without coupling [10]. The coupling strength K would be related to the amount of protoplasm transported by the protoplasmic streaming through the tube structure. We assumed the relation $K = -c \cdot \pi[D(W)/2]^2 V_{\text{proto}}(W, L)$, where $D(W)$ is the tube diameter depending on W [11], $V_{\text{proto}}(W, L)$ is the time averaged velocity of the protoplasmic streaming in its absolute value, which is depending on W and L [13], and c $(= 3.8 \times 10^{-5})$ is an arbitrary coefficient. The changes in the amount of protoplasm caused by the thickness oscillation was on the same order as those due to the protoplasmic streaming according to our calculations

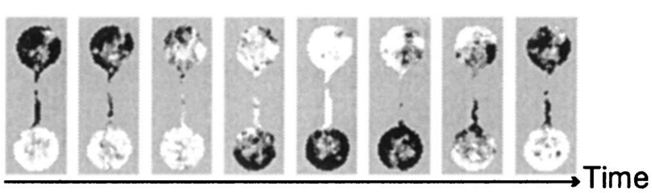

FIG. 2. Thickness oscillation in the two oscillator system. $W = 0.40$ mm; $L = 4$ mm. Black/white images indicate increase/decrease in thickness. The time intervals between these images were 16 sec. The thickness data were obtained from the transmitted light intensity through the plasmodium at intervals of 4 sec. We have confirmed that the light intensity is proportional to the thickness [5]. The experiments were performed in the thermostat and humidistat chamber (PR- 2K, ESPEC) under the condition at 25 ± 0.3 °C and RH85 ±2.5% to reduce the effects on the oscillation frequency, which were ±0.001 and ±0.002, respectively, in our experimental setup. They are small enough against the stepwise frequency changes in Fig. 5.

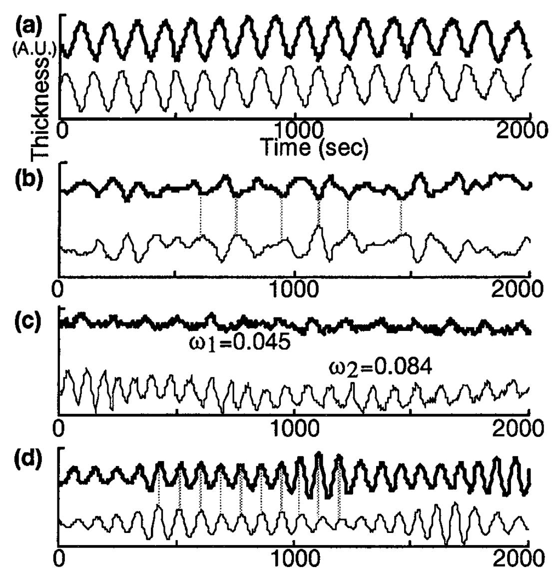

FIG. 3. Time course of thickness oscillation. The thick and thin lines represent the thickness in individual oscillators that was obtained by taking averages of the transmitted light images from each oscillator part. (a) Antiphase: $W = 0.40$ mm; $L = 4$ mm. (b) Quasiperiodic antiphase: $W = 0.20$; $L = 4$. (c) Unentrained: $W = 0.10$; $L = 4$. (d) In-phase oscillation: $W = 0.50$; $L = 10$.

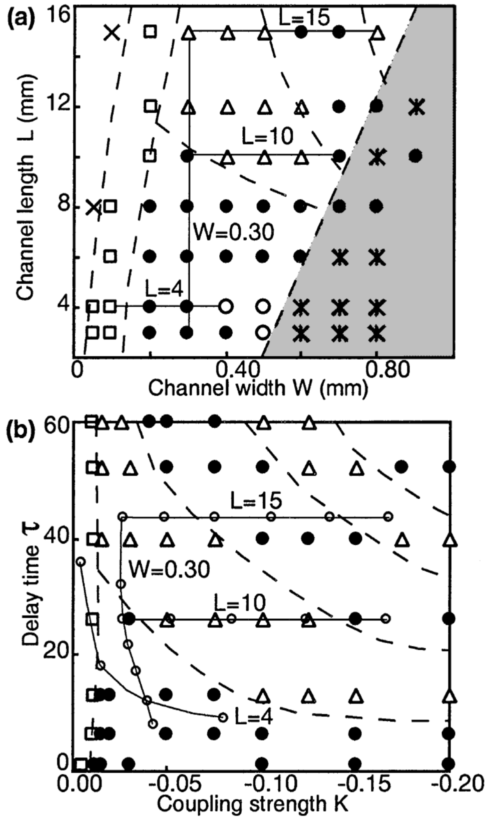

FIG. 4. Phase diagrams. (a) Experimental results on the W-L plane. Squares mean that although the two oscillators were physically connected by the protoplasmic streaming, they were not entrained. Filled circles and open circles mean quasiperiodic antiphase and antiphase, respectively. See text about the meanings of the other marks. (b) Numerical results obtained from Eqs. (2) and (3) on the K-τ plane. Here we set the intrinsic frequency as $\omega_1 = 0.05$, $\omega_2 = 0.07$. Squares, circles, and triangles mean unentrained (the equations have no solution), antiphase ($\alpha = \pi$), and in-phase ($\alpha = 0$) oscillation, respectively. The thin lines with small open circles correspond to the thin lines in (a).

[14]; namely, in short distance, the protoplasmic streaming would directly affect the change in the thickness of the oscillators so that the contraction in one oscillator would cause the expansion in the other oscillator. Thus we assumed $K < 0$.

The time delay could also affect the coupled plasmodial oscillator system. The duration of the wave propagation from one oscillator to the other is estimated, for example, at about 27 sec when $L = 10$ mm [15]. This is not negligible against a period of oscillation (about 100 sec). Here, we estimated the delay time τ from the wave propagation velocity in the tube $V_{\rm wave}(W, L)$ [15] by the relation $\tau = (L - a)/V_{\rm wave}(W, L)$, where a (= 2 mm) is the radius of the oscillator part.

Schuster and Wagner solved Eqs. (1) under the condition $K > 0$ to obtain multistable phase locked solutions and investigated the stability of those solutions. Under the condition $K < 0$, phase locked solutions of common frequency $\Omega(K, \tau)$ and phase difference α in Eqs. (1) are determined by the following equation that can be derived by the same method by Schuster and Wagner [2]:

$$\omega_0 - \Omega + K \tan(\Omega\tau)\sqrt{\cos^2(\Omega\tau) - \Delta\omega^2/K^2} = 0, \tag{2}$$

$$\begin{aligned} \alpha &= \arcsin[\Delta\omega/K\cos(\Omega\tau)] \quad \text{if } \cos(\Omega\tau) < 0, \\ &= \pi - \arcsin[\Delta\omega/K\cos(\Omega\tau)] \quad \text{otherwise,} \end{aligned} \tag{3}$$

where $\omega_0 = (\omega_1 + \omega_2)/2$ and $\Delta\omega = (\omega_1 - \omega_2)/2$. Note that the differences from the case of the condition $K > 0$ are the sign of the third term on the left side of Eq. (2) and the conditions of $\cos(\Omega\tau)$ for Eqs. (3).

We calculated the most stable solutions from the multiple stable solutions [2]. Figure 4b shows the phase diagram on the K-τ plane obtained from the numerical results of Eqs. (2) and (3). This phase diagram is similar to that from the experimental results, namely, there are three following features, unentrained, antiphase, and in-phase oscillations; moreover, the phase transitions between antiphase and in-phase repeated as the parameters increased.

Another interesting characteristic of the time delayed system is that the common frequency Ω changes stepwise at the phase transition. Figure 5 shows the experimentally and numerically derived values of the angular frequencies of oscillation when W or K increases along the thin lines in the phase diagrams (Fig. 4). We observed similar phenomena such as the stepwise changes of Ω in both the experimental and the theoretical results. In Figs. 5a and 5d, the most remarkable characteristics of the delayed system can be observed, where Ω decreases as K and τ increase (note that, in the case of K, such phenomena are expected only before the first transition in the theory [2,3]). However, Ω increased after it once decreases in Fig. 5d. This would be due to the fact that, in our experiment, K could not be kept constant along with the line of $W = 0.30$ (mm) as shown in Fig. 4b, then K slightly shifted smaller as τ increased. Around the transition points, the plasmodial system demonstrated intermittent spontaneous switching between in-phase and antiphase during serial experiments; these findings would correspond to the multistable solutions found in the theoretical results [2–4]. The change manners of Ω in experimental results were slightly different from those in theory as shown in the IP region of Fig. 5b and the AP region of Fig. 5c, where experimental values decreased whereas theoretical ones increased. From such minor differences, it would be possible to ascertain more detailed information about the dynamics of the oscillator itself or the coupled oscillator system in the plasmodium by comparing them with modified models such as those proposed by a number of theorists [4] or by proposing a new model.

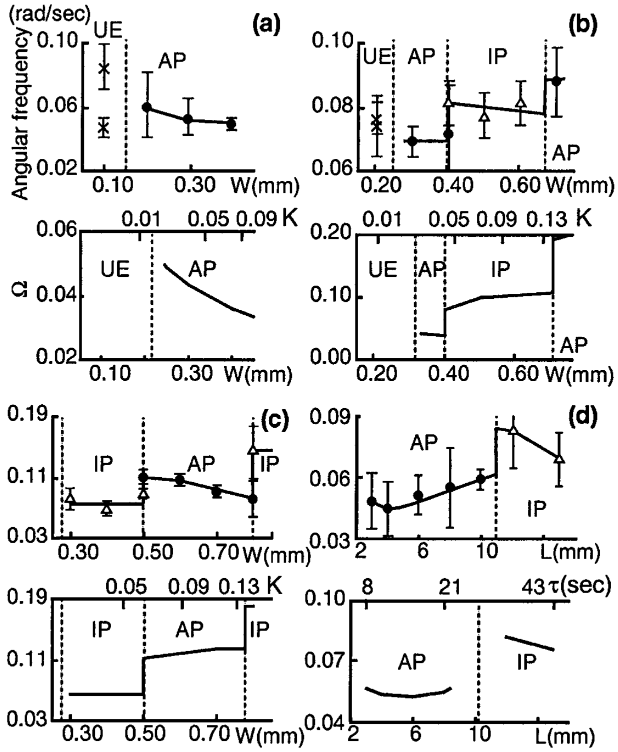

FIG. 5. Angular frequency. Upper graphs were obtained from the experimental results by averaging the angular frequencies over ten periods of the oscillation at every data point. Lower graphs depict the calculated values from the most stable solution of Eqs. (2) and (3). UE, AP, and IP mean unentrained, antiphase, and in-phase oscillation, respectively. (a) $L = 4$ mm. (b) $L = 10$ mm. (c) $L = 15$ mm. (d) $W = 0.30$ mm. The data in (a), (b), (c), and (d) correspond to those of the thin lines in Fig. 4.

In conclusion, we demonstrated the time delay effect in the plasmodial slime mold by using the living coupled oscillator system and by comparing our experimental results with those of the theoretical analysis based on a simple mathematical model. Now we become interested in whether or not the amplitude death [1] exists in such a system. In the plasmodial system, the amplitude death does not exist, because the plasmodium in the wide channel forms a dendritic tube structure (Fig. 1e) so that the coupling strength never increases beyond a certain W [11], although strong coupling is one of conditions for the death. This phenomenon might be advantageous to the plasmodium, where it prevents the risk of death.

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[11] The critical channel widths, whether a single tube structure or a dendritic one is formed, are $W = 0.40$, 0.70, and 0.80 (mm), when $L = 4$, 10, and 15 (mm), respectively. We measured the single tube diameter D (mm) from its digital image under a microscope. D can be approximated by the relation $D = (-1.47W^2 + 3.15W) \times 10^{-1}$, independently of L [12].
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[13] The velocity of the protoplasmic streaming was determined by measuring the time required that organelles observed in the tube structure stream a certain distance on the video monitor [12]. Then we calculated the time averaged velocity in its absolute value, V_{proto} (mm/sec), which depended on both W (mm) and L (mm), according to the approximate relations, $V_{\mathrm{proto}} = 6.16W \times 10^{-1}$ ($L = 4$ mm; $W = 0.10-0.40$ mm), $V_{\mathrm{proto}} = (2.46W + 0.41) \times 10^{-1}$ ($L = 10$; $W = 0.30-0.70$), $V_{\mathrm{proto}} = (1.50W + 0.71) \times 10^{-1}$ ($L = 15$; $W = 0.30-0.80$), and $V_{\mathrm{proto}} = (0.01L^2 - 0.26L + 2.72) \times 10^{-1}$ ($W = 0.30$).
[14] For example, the amount transported by the protoplasmic streaming is estimated to be about 0.1 $\mu\ell$ per half of a period of the oscillation when $L = 4$ mm, the velocity is 0.25 mm/sec, and a period of oscillation is 125 sec. The amount changed by the thickness oscillation in the oscillator part (the change in thickness was ± 0.1 mm in our observations) is estimated to be about 0.3 $\mu\ell$.
[15] The wave propagation velocity V_{wave} (mm/sec) was determined by observing the increasing wave of thickness in the coupling part of the plasmodium and by measuring the distance that the wave front moved in a certain duration. The velocity depended on W (mm) and L (mm) according to the approximate relations, $V_{\mathrm{wave}} = 5.61W \times 10^{-1}$ ($L = 4$ mm; $W = 0.10-0.40$ mm), $V_{\mathrm{wave}} = 3.07 \times 10^{-1}$ ($L = 10$; 0.30–0.70), $V_{\mathrm{wave}} = 2.98 \times 10^{-1}$ ($L = 15$ $W = 0.3-0.8$), and $V_{\mathrm{wave}} = (-0.25L^2 + 5.86L - 2.57) \times 10^{-2}$ ($W = 0.30$).