J. theor. Biol. (1988) **131**, 175–182

Reversal of Thermotaxis with Oscillatory Stimulation in the Plasmodium of *Physarum polycephalum*

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(*Received* 25 *August* 1987)

The plasmodium of *Physarum polycephalum* shows shuttle protoplasmic streaming and, upon steady local stimulation, moves toward or away from high or low temperatures, respectively. However, these thermotactic behaviors were reversed by oscillating the stimulus temperature. When the high temperature stimulus (originally an attractant) was oscillated slower than the intrinsic rhythm, the organism moved away from there as long as the entrainment was maintained. On the other hand, low temperature (a repellent), when oscillated faster to entrain the rhythm, attracted the organism. The implication of this result is discussed in relation to the mechanism of information processing in the plasmodium in terms of a coupled oscillator model.

Introduction

The giant amoeboid cell of the *Physarum* plasmodium is useful for studying the intracellular mechanism of information processing which brings about co-ordination in the cell behavior (Ueda & Kobatake, 1982). The organism extends like a sheet at the frontal part, and intricate veins develop toward the rear, in which the protoplasmic sol moves to and fro very regularly due to the difference in hydrostatic pressure (Kamiya, 1959). Accompanying this rhythmic contractility, the thickness of the protoplasm also oscillates with period of 1–2 minutes. We have analyzed the dynamic behavior of the intrinsic rhythm with the help of computer image processing for the thickness oscillation. When a part of the plasmodium is stimulated with temperature, chemicals or light, waves of thickness oscillation propagate throughout the organism, the phase around the stimulated region being advanced or retarded for attractants or repellents, respectively (Matsumoto *et al.*, 1986; Ueda *et al.*, 1986; Mori *et al.*, 1986). This suggests that the intracellular information processing within the plasmodium is related to a dynamic cooperative behavior in a population of coupled non-linear oscillators. Frequency modulation seems to play an important role here, because attractants and repellents make the intrinsic rhythm of the organism fast and slow, respectively (Durham & Ridgway, 1976; Tso & Mansour, 1975; Block & Bottermann, 1981).

Non-linear oscillators have the ability to entrain to oscillatory external stimuli, as demonstrated both experimentally and theoretically (Kuramoto, 1984; Winfree, 1980). If this is true for the intrinsic rhythm of the *Physarum* plasmodium, we will be able to modulate the frequency of the intrinsic rhythm by oscillating the external factors. And if the frequency modulation plays a key role in intracellular information processing, the behavior of the organism will also be modified.

0022-5193/88/060175+08 $03.00/0

In this paper we test this idea for thermotaxis in the *Physarum* plasmodium, because temperature can be controlled with ease, and also because it may affect the kinetic parameters rather than the concentration of metabolites themselves.

Material and Methods

ORGANISM

The plasmodium of *Physarum polycephalum* (HU 520 × Hu 525) was fed with oatflakes on an agar gel. The organism was allowed to move on 1% water-agar in a trough ($22 \times 33\ cm^2$) overnight, thus affording a large migrating plasmodium. Several pieces of the tip portion of this organism were placed on a new water-agar in a measuring apparatus and a plasmodium extended concentrically a few hours later.

LOCAL STIMULATION OF THE ORGANISM WITH OSCILLATING TEMPERATURE

The apparatus (Fig. 1a) was constructed to stimulate a small square region (ca. 1×2 cm) of an extending plasmodium with oscillating temperature. The stimulus temperature was oscillated by pumping water alternately from two heat baths maintained at different temperatures T_1 and T_2, at appropriate time intervals under microcomputer control (Fig. 1b). Temperatures on the surface of the agar gel were measured with IC temperature sensors (Analog Divices, AD590) and recorded in a 16-bit microcomputer (NEC, PC9801E) through an A/D converter. The temperature oscillated almost sinusoidally somewhere between T_1 and T_2 (see Figs 5 and 6).

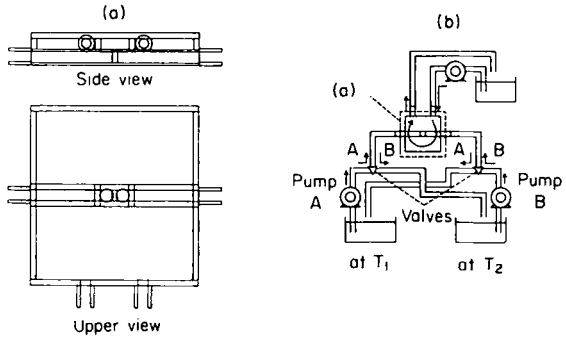

FIG. 1. Schematic illustrations of experimental set-ups. (a) The apparatus (plexiglass) used for oscillatory local temperature stimulation. Temperature at a square region (ca. $1 \times 2\ cm^2$) near the center was varied. (b) A block diagram for oscillatory temperature variation. Waters thermostated at T_1 and T_2 were circulated alternately at appropriate time intervals by two pumps (A and B) controlled by a microcomputer, while the temperature of the surroundings was kept constant by circulating thermostated water continuously.

COMPUTER IMAGE PROCESSING

Dynamic behavior of the intrinsic rhythm (thickness oscillation) in the *Physarum* plasmodium was analyzed by computer image processing. Plasmodia migrating on agar were illuminated from below through frosted glass with yellow light emitting diodes. Pictures were taken from above with a TV camera and recorded by a videorecorder. Video-images were digitized (by ALTEC SYSTEM, ALT256-8-4DMA video digitizer) at 4-sec and intervals, then transferred to a 16-bit microcomputer (NEC, PC9801VM) and processed. Original 8-bit intensity data at 256×256 points were summed over a square of 4×4 points to give 16-bit data at 64×64 points, thus saving both computer memories and computing time.

From original time series of the light intensity at a given point (Fig. 2), we separated slow and fast components. The slow one, which was obtained by averaging over a few period (240 sec), is a measure of the net movement of the protoplasm and hence a tactic behavior (A in Fig. 2): an increase in the light intensity at a point indicates a decrease in thickness there, and hence avoidance; and *vice versa.*

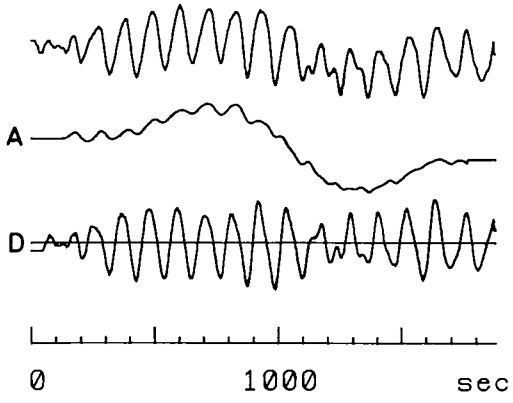

FIG. 2. Separation of slowly drifting and fast oscillating components from thickness variation. From an original time course of the thickness variation, a time course of slowly drifting components was obtained by averaging over 240 sec (A), while that of fast oscillating components was obtained by taking a difference between values at time t and t-40 sec (D). The former reflects the averaged protoplasmic movements and hence the tactic behavior and the latter are used for determining the oscillation patterns as shown in Fig. 3.

The fast component was obtained by taking a difference between the data at time t and the previous time $t - t_1$ (D in Fig. 2). We used $t_1 = 40$ sec (about 1/3 of the period), because this gave the largest signal to noise ratio. This difference time series was used for determining oscillation patterns as shown in Fig. 3, where the brightness level corresponds to the difference value of the time series. Upon local stimulation, a metachronal wave appeared around the stimulated region, the phase there being advanced or retarded to high (attraction) or low (repulsion) temperatures, respectively. In order to determine changes in the phase relationship with time, these 2-D phase relationships were simplified to a 1-D version by taking data along a diameter

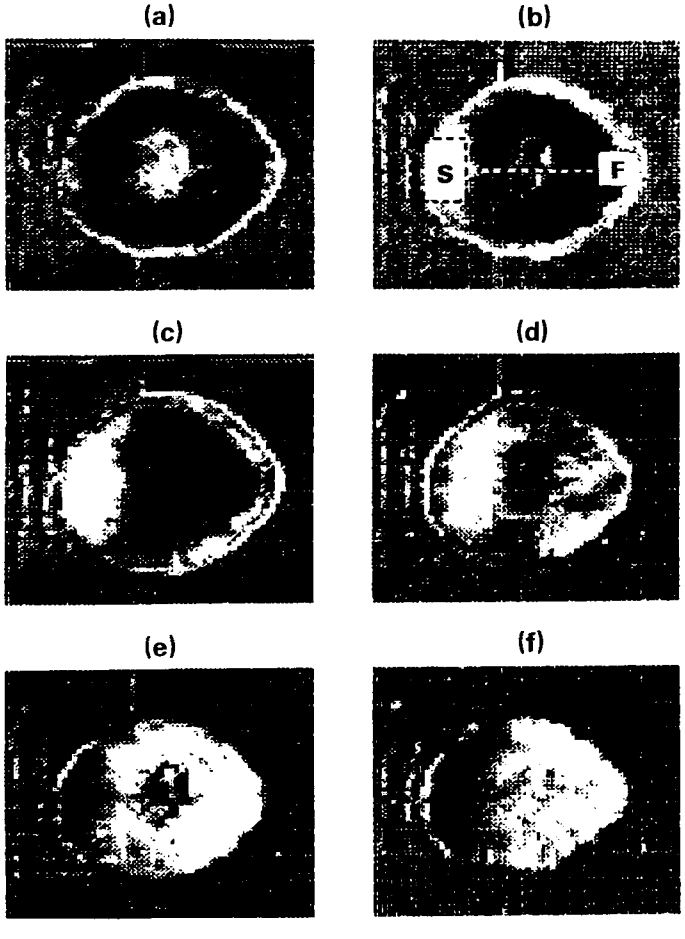

FIG. 3. Oscillation patterns after local temperature stimulation. Oscillation patterns are at 0 (a), 16 (b), 32 (c), 48 (d), 64 (e) and 80 (f) sec and 5 min after local stimulation. 1-D patterns connecting the stimulated (S) and unstimulated (F) regions are displayed successively, giving patterns (P) as shown in Figs 4–6.

from the stimulated end S to the other end F. These were arranged vertically and depicted successively with time, giving patterns as shown in Figs 4–6.

Experiments were repeated at least 5 times at each experimental condition, and results reported here are typical.

Results

RESPONSES OF THE PLASMODIUM TO STATIONARY STIMULATION WITH TEMPERATURE

Figure 4 shows time courses of the response when the organism was stimulated locally with stationary high temperature. The middle two traces are time courses

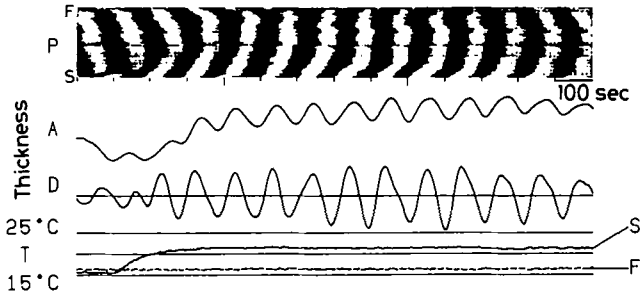

FIG. 4. Time courses of thickness and phase relation when stimulated with constant high temperature. T: time courses of temperature at stimulated (S) and unstimulated (F: free) points. Middle trace: time courses of oscillating (D: difference) and drifting (A: averaged) components at stimulated point (S). P: variation of phase relationships between stimulated and unstimulated points with time. The pattern becomes right-up upon stimulation (the phase at S advances) and the thickness at S increases rapidly (positive thermotaxis).

of fast and slow components at the stimulated point S, respectively. The top is a time course of 1-D oscillation pattern. Variations in temperature at points S and F are shown at the bottom. Upon stimulation with high temperature, the protoplasm moved toward the stimulated region and the thickness there became larger and larger. As seen in the top patterns, the phases at the stimulated region became advanced and entrained the neighboring part. All these features are common in response to attractive stimuli (Matsumoto *et al.*, 1986). On the other hand, when stimulated with low temperature, the organism moved away from there, the phase there being retarded (data not shown here). This is also a common feature in response to repellents.

MODIFICATION OF RESPONSES TO HIGH TEMPERATURE BY OSCILLATING THE STIMULUS TEMPERATURE

Responses to temperature as described above are reversed by oscillating the stimulus temperature. Figure 5 shows a typical response of the organism to oscillatory

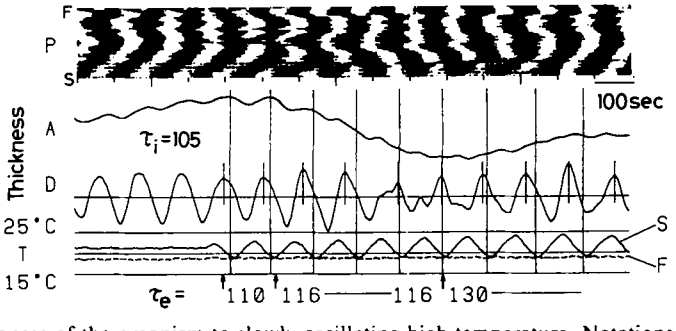

FIG. 5. Responses of the organism to slowly oscillating high temperature. Notations are the same as in Fig. 4. τ_e indicates periods (in sec) of the forced oscillation, τ_i the period of the intrinsic rhythm of the organism. Positive thermotaxis to high temperature became negative as long as the intrinsic rhythm of the organism entrained into the forced slow oscillation.

stimulation where the stimulus temperature was always kept higher than the surrounding constant temperature. In this case, the phase at the point S before stimulation happened to advance the phase at the point F, and the thickness at the point S tended to increase gradually. However, when the stimulus temperature was oscillated a little slower than the intrinsic rhythm of the organism, the rhythm at the stimulated part entrained to this external oscillation. As a result, the phase at point S became retarded. At the same time, the protoplasm began to move away from the stimulated region. Namely, the organism moved away from the high temperature region. Namely, the organism moved away from the high temperature (originally attractant) when slowly oscillated. But, when the frequency of the temperature oscillation was lowered to such an extent that the intrinsic rhythm no longer entrained, the phase at the stimulated region became advanced as before the onset of oscillation and the protoplasm moved toward the region stimulated with, on average, high temperature. Thus, the behavior is reversed as long as the entrainment is maintained. The organism responded to fast high-temperature oscillation in the same way as with stationary stimulation (Fig. 4).

MODIFICATION OF RESPONSES TO LOW TEMPERATURE

The organism moves away from the low temperature, but this thermotaxis was also reversed. Responses of the organism to low temperature are shown in Fig. 6 where stimulus temperature was oscillated slightly faster than the intrinsic rhythm and always kept lower than the surrounding temperature. Before the oscillation began, the phase at the region retarded. The external oscillation with period of 100 sec, entrained the rhythm of that part which further entrained the neighboring rhythm, leading to the advanced phase at the stimulated part. The moment this phase relation was established, the protoplasm moved toward the stimulated region: the negative taxis to low temperature becomes positive with oscillatory stimulation. When the stimulated part was no longer entrained by the forced oscillation, the phase at the stimulated region retarded again and the protoplasm moved away from there.

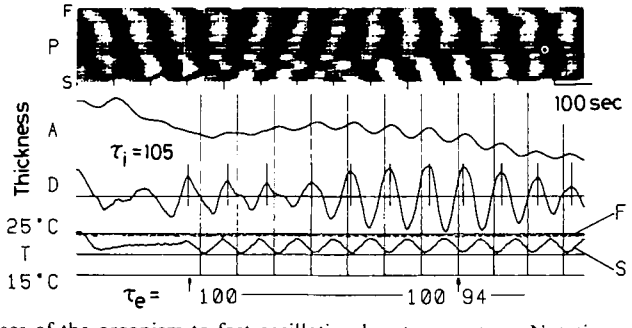

FIG. 6. Responses of the organism to fast-oscillating low temperature. Notations are the same as in Fig. 5. Negative thermotaxis to low temperature became positive as long as the rhythm of the organism entrained into the fast oscillation.

No modulation took place when low temperature was oscillated slowly: the phase wave propagated toward the stimulated region and the protoplasm moved away from there.

Discussion

The results shown in Figs 5 and 6 indicate that the metachronal phase relation is determined solely by the distribution of oscillators with different periods, the phase on the side of intrinsically faster rhythm being advanced, and that the net movement of the protoplasm occurs along the direction of phase gradient toward the region where the phase advances. The latter situation has also been demonstrated in response to various external stimuli (Matsumoto *et al.*, 1986; Mori *et al.*, 1986).

A coupled oscillator model seems appropriate to interpret the results (Winfree, 1980; Ermentrout & Troy, 1986). Let us regard the plasmodium as a population of oscillators which are fixed on the membrane and distributed uniformly. The oscillators interact with each other indirectly through the moving protoplasmic sol, and thus a delay in the interaction is unavoidable. These oscillators are so coupled to the motile system as to generate the motive force for the protoplasmic flow as they complete a cycle. The nature of the oscillator seems to be metabolic and coupled to membrane transport, because ATP (Yoshimoto *et al.*, 1981a), cAMP (Ueda *et al.*, 1986), NADH (Mori *et al.*, 1987), H^+ (Nakamura & Kamiya, 1985) and Ca^{2+} (Yoshimoto *et al.*, 1981b) all oscillate accompanying the rhythmic contraction. Also, studies using metabolic inhibitors have shown that mitochondria and glycolysis play a part in the cellular oscillator (Satoh *et al.*, 1982, 1985; Korohoda *et al.* 1983; Baranowski, 1985).

Based on this model, we can outline the mechanism of information processing in the *Physarum* plasmodium: (1) both the kind and the strength of stimulation are encoded into the period of local oscillators (frequency modulation), because the period of the oscillation became uniformly shorter as the temperature was raised uniformly; (2) a change in the period of these oscillators induces an overall change in the phase relation throughout the plasmodium, whereby either the faster oscillators entrain the lower ones, or they entrain mutually; and (3) the resulting metachronal phase relation in a population of oscillators governs the net movement of the protoplasm.

As for the molecular basis of the spatial organization, intracellular ATP distribution is shown to be related to the directed movement of the plasmodium (Ueda *et al.*, 1987), but its relationship with the propagation of the metachronal wave has yet to be clarified.

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