Amoebae Anticipate Periodic Events

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When plasmodia of the true slime mold *Physarum* were exposed to unfavorable conditions presented as three consecutive pulses at constant intervals, they reduced their locomotive speed in response to each episode. When the plasmodia were subsequently subjected to favorable conditions, they spontaneously reduced their locomotive speed at the time when the next unfavorable episode would have occurred. This implied the anticipation of impending environmental change. We explored the mechanisms underlying these types of behavior from a dynamical systems perspective.

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Information processing is an interesting component of biological systems. Although the brain has evolved to perform this specific function, information processing is possible without a brain, and organisms as simple as amoebae are much more intelligent than generally thought. For example, the true slime mold *Physarum polycephalum* can solve a maze and certain geometrical puzzles, in order to satisfy its needs for efficient absorption of nutrients and intracellular communication [1–4]. Thus, from an evolutionary perspective, information processing by unicellular organisms might represent a simple precursor of brain-dependent higher functions. Anticipating and recalling events are two such functions; however, the way in which they self-organize has so far remained unknown.

P. polycephalum is a useful model organism for studying behavioral intelligence [5]. Its plasmodium is a large aggregate of protoplasm that possesses an intricate network of tubular structures, and can crawl over agar plates at a speed of approximately 1 cm/h at room temperature. In order to investigate primitive forms of brain function (such as learning, memory, anticipation, and recall), here we have examined the rhythmicity of cell behaviors [6,7] and the adaptability of cells to periodic environmental changes [8,9]. Our approach was to expose organisms to periodic changes in ambient conditions and to observe their behavioral responses. As there has been much controversy in recent years over the existence of nonhuman intelligence [10–13], this subject requires evaluation by modern scientific techniques.

Here we show that an amoeboid organism can anticipate the timing of periodic events. Moreover, we explore the mechanisms underlying this behavior from a dynamical systems perspective. Our results hint at the cellular origins of primitive intelligence, and imply that simple dynamics might be sufficient to explain its emergence.

A large plasmodium of *P. polycephalum* was cultured with oat flakes in a trough (25×35 cm^2) on an agar plate under dim light. The tip region of an extended front was removed and placed in a narrow lane (0.5×28 cm^2) at 26 °C and 90% humidity (hereafter referred to as standard conditions; all experiments were conducted under these ambient conditions unless otherwise specified). The organism migrated along the lane, and the position of its tip was measured every 10 min. After migration had been permitted for a few hours, the ambient conditions were changed to cooler (23 °C) and drier (60%) (referred to as ''dry stimulation'') conditions for 10 min as shown in Fig. 1(a). This dry stimulation was repeated 3 times at various intervals of τ (the values of τ tested were 30, 40, 50, 60, 70, 80, and 90 min). The experiments described above were performed in an incubator (Type KCL-10000, Eyela Co.), and the temperature and humidity were controlled. The organism moving along the lane was illuminated from below by a matrix of infrared light and was viewed with a charge-coupled device (CCD) camera. The window of the incubator was covered with a cutoff filter in order to transmit only infrared light, so that the experimental material was kept in the dark.

Figures 1(a)–1(c) show a typical time course of locomotion speed and acceleration, along with temperature and humidity. Locomotion speed decreased during the periods of dry stimulation ($\tau = 60$ min) at time points $S1$, $S2$, and $S3$. After the dry stimulation, standard conditions were maintained; however, the movement slowed spontaneously at time points $C1$, $C2$, and $C3$ [closed arrows in Figs. 1(b) and 1(c)], which coincided with the time points when the next periods of dry stimulation would have occurred. This slowing of locomotion, which is referred to as spontaneous in-phase slowdown (SPS), occurred at the first and second time points after the last true dry stimulation.

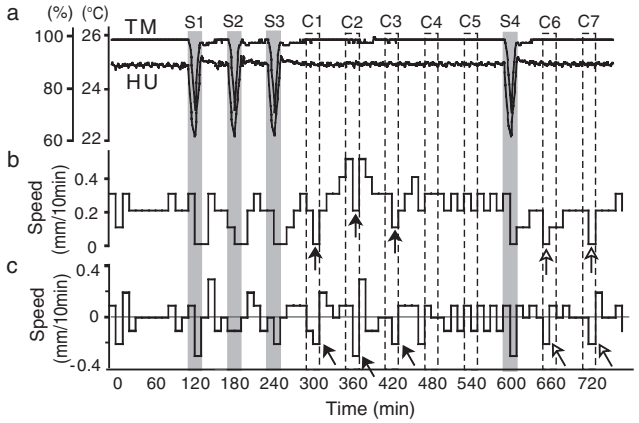

FIG. 1. SPS and SPSD responses. Typical time course of locomotion for an organism (wet weight = 15 mg) before and after three periodic applications ($S1$, $S2$, and $S3$) and single application ($S4$) of dry stimulation. (a) Temperature is denoted by the upper line (TM) and humidity by the lower line (HU). (b) Locomotion speed calculated over successive 10 min intervals. (c) Acceleration (Δ speed) defined as the difference in speed between successive intervals in (b). $S1$, $S2$, $S3$, and $S4$ indicate the time points of the real stimulations, whereas $C1$, $C2$, ..., $C7$ indicate the time points of the virtual stimulations. The SPS response was induced at time point $C1$, $C2$, and $C3$ by periodic stimulation (closed arrows), and at time point $C6$ and $C7$ by trigger stimulation at $S4$, after disappearing once (open arrows).

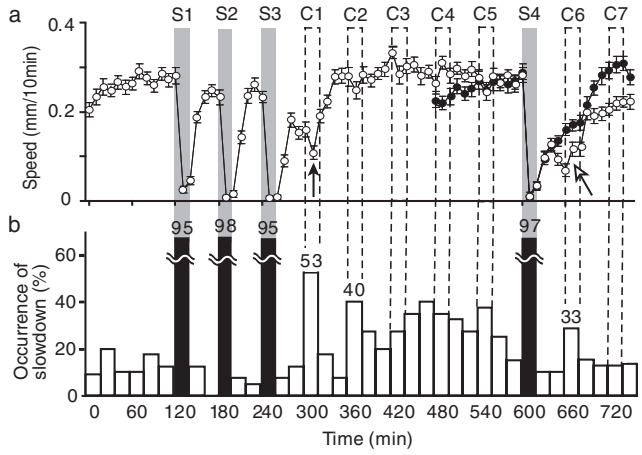

FIG. 2. Statistical analysis of the SPS and SPSD responses in terms of mean speed with standard error (a) and statistical occurrence of spontaneous slowdown (b). The mean speed was calculated from 43 examples for the tested group (open circles), and from 39 samples for the control group with no in-advance periodic stimulation (closed circles). Points $S1$–$S4$ indicate the times of the real stimulations, whereas points $C1$–$C7$ indicate the times of the virtual stimulations. The SPS response was induced at $C1$ by periodic stimulation (closed arrow), and at $C6$ by trigger stimulation at $S4$, after disappearing once (open arrow). The organisms had weights ranging from 10 to 19 mg.

Figure 2 shows the results of statistical analyses of the SPS, indicating the average speed [Fig. 2(a)] and statistical occurrence of slowdowns [Fig. 2(b)], calculated over 43 repeats. The locomotion speed dropped significantly at the first instance of virtual dry stimulation. SPS was evident at this point in the averaged time course, and the analysis revealed the SPS at $C1$ more clearly [closed arrow in Fig. 2(a)]. The initial wet weights of the organisms in Fig. 2 were 10–19 mg. The smaller plasmodia, in the range of 5–10 mg wet weight, were more sensitive to the dry stimulation, and those below 5 mg wet weight could be seriously damaged by it. The SPS response was thus dependent on the size of the plasmodium.

Next, the τ value of the environmental changes was varied from 30 to 90 min. Figure 3 shows the statistical occurrences of SPS under these conditions. At $\tau = 60$ min, SPS was exhibited once by 30% of the organisms, twice by 20%, and 3 times by 10%, while 40% of the organisms failed to undergo SPS. Instances of SPS were evident for all the values of τ tested. The overall frequency of SPS was approximately 40%–50%, and this figure was roughly similar for all periods. Time periods shorter than 30 min or longer than 90 min were not tested due to technical limitations of our experimental setup.

After a few occurrences of SPS at $\tau = 60$ min, the locomotion speed fluctuated and no further SPS was observed. However, as shown in Fig. 1, SPS reappeared at $C6$ and $C7$ [open arrows in Figs. 1(b) and 1(c)] in response to a single dry stimulation at $S4$. This phenomenon is referred to as SPS after one disappearance (SPSD). SPSD was examined through a statistical analysis of the average speed [Fig. 2(a)] and the occurrence of slowdown [Fig. 2(b)]. The occurrence of SPSD was clearly observed [open arrow in Fig. 2(a)]. When the time point of $S4$ was varied from 420 min to 600 min, the occurrences of slow-

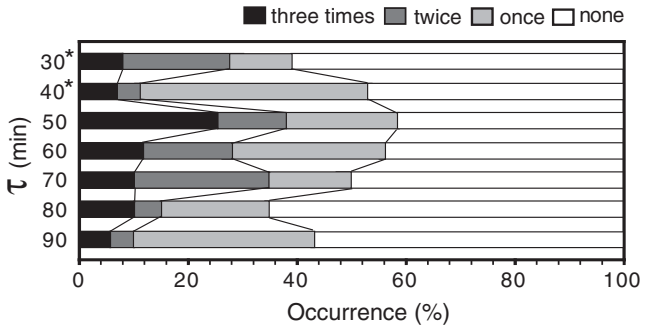

FIG. 3. SPS responses over a range of stimulation periods (τ). The gray levels of the bars indicate the number of occurrences of SPS: black denotes three, dark gray denotes two, light gray denotes one, and white denotes none. The twofold criteria for SPS were that there should be a unimodal evolution of locomotion speed between the last stimulation and the time of the subsequent virtual stimulation, and that the minimum locomotion speed should occur at the time of this virtual stimulation. Bimodal time courses and fluctuations of locomotion speed were often observed, and were regarded as an absence of SPS, as were instances when the minimum speed occurred a little earlier or later than the virtual stimulation. *: Dry stimulation at 26 °C and 70% humidity, which is a weaker stimulus than in all other cases. Number of repeats (τ min): 36 (30), 46 (40), 24 (50), 121 (60), 20 (70), 20 (80), and 19 (90).

down at the first virtual stimulation after trigger stimulation at $S4$ were 63% ($S4$ at 420 min), 39% (480 min), 40% (510 min), 39% (570 min), and 33% (600 min). At all time points tested, SPSD was significant. Note that the time points of 510 min and 570 min are antiphase of the previous periodic stimulation. This SPSD was observed at $\tau = 60$ min, but not 40 min and 80 min. The SPSD response was thus limited at a specific period.

We have developed a dynamical system model that reproduces the observed phenomena. Figure 4 shows a model simulation and schematic illustration of the behaviors. The model is based on physiological observations. We assume that multiple chemical oscillators of a series of periods underlie the multirhythmicity of locomotion, as multiple rhythms were observed in cellular activities in a *Physarum* plasmodium [6,7]. These cell movements showed a $(\text{frequency})^{-1}$-type property of power spectrum density in a frequency range from 1 s to 24 hours. This means that there are continuous frequencies of oscillation. If variations of power (amplitude) through the frequency range are neglected, these might be described by simple phase oscillators of the type $d\theta_{i,j}/dt = \omega_j$ $(0 \leq \theta < 1)$, where $\theta_{i,j}$ is the phase of an oscillator of a frequency ω_j. The multirhythmicity shown in a small portion of the protoplasm is modeled by a collection of oscillators with different periods indicated by j.

As the plasmodium consists of a large amount of protoplasm, there should be many oscillators numbered i at each frequency ω_j. For this oscillator group to respond to the external periodic dry stimulation, the dynamics can be written as

$$\frac{d\theta_{i,j}}{dt} = \omega_j + \alpha H(t)\sin(2\pi\theta_{i,j}) + \xi_{i,j}, \qquad (1)$$

where the second and the third terms on the right are the periodic stimulation and random noise, respectively, and $H(t)$ is responsible for the expression of a periodicity ω_{ext} as shown in Fig. 4(a). As $H(t) = 1$ in the presence of dry stimulation unless otherwise $H(t) = 0$. The second term of the periodic stimulation acts to reduce $\cos\theta$ whenever $H(t) = 1$, so that the activity level of locomotion is assumed to be proportional to $\cos\theta$.

The total activity S of locomotion over an entire organism then depends on the sum of oscillations as

$$S = \sum_j \tanh\left(2\sum_i^N \frac{\cos 2\pi\theta_{i,j}}{N} + 3\right), \qquad (2)$$

where tanh comes from the experimental observation that locomotion speed is saturated at high intracellular concentrations of some important chemical component [14]. Both SPS and SPSD responses are reproduced by the model equations, as shown in Fig. 4(a).

The total activity of locomotion S does not display any oscillatory variation before the single trigger stimulation is applied, as it is similar to the state before the periodic stimulation. However, the internal state of the collective

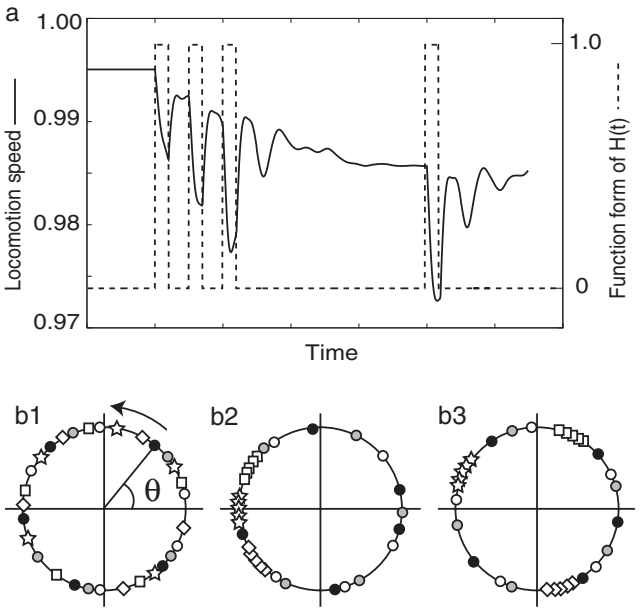

FIG. 4. Simple dynamics model and simulation of SPS and SPSD responses. (a) Simulation of the variation of locomotion speed S with time (black line), and the function form of $H(t)$ (dashed line). (b) Schematic illustration of the model behaviors. Many oscillators with different frequencies (the same symbols denote the same frequencies) rotate counterclockwise on the ring in the state space spanned by two kinds of chemical species that are not specified and might be any chemicals. In the initial conditions the phase θ of the oscillator is distributed at random; no coherent motion is observed, so that locomotion speed S is nearly one (b1). After periodic perturbations of frequency ω_{ext} are applied to all the oscillators, the multiple oscillators with $\omega_j = \omega_{\text{ext}}$ (open stars) tend to synchronize because they are pushed leftward on the ring during the perturbation (b2). This synchronization is observed in oscillators with each frequency of $\omega_j \simeq \omega_{\text{ext}}$ (open squares and open diamonds). At this point, S shows clear periodic variations with the same frequency as the external perturbations, and this state is maintained until the coherent oscillators desynchronize in phase, due to differences between their intrinsic frequencies ω_j (b3). Note that groups of oscillators of the same frequency remain synchronized even though S does not show any clear periodic behavior. When the perturbation is applied again, the oscillator groups of the same frequency tend to synchronize in phase, so that S again shows periodic behavior (b2). $\omega_j = 0.1, 0.11, 0.12, 0.13, \ldots, 4.49, 4.50$; $N = 1000$; $\alpha = 0.5$; $-0.001 < \xi < 0.001$.

oscillators is different as some coherent groups of oscillators persist, as shown in Fig. 4(b)3. Even though the appearance of the total activity is similar, it is possible to generate a different internal state within the system. It is also predicted theoretically that the relaxation of total activity does not always imply decay of the internal state; in a large population of coupled oscillators, the latter takes a much longer time than the relaxation of total activity [15]. Although our model is not identical to the model analyzed in the previous paper, the model behaviors described above resemble the theoretical prediction. The

hidden order of the internal state might play a key role in the self-organization of SPS and SPSD observed in the real organism. SPS and SPSD can therefore be accounted for by these simple dynamics.

It should be noted that our model does not involve interactions between the oscillators. The random noise $\xi_{i,j}$ is not necessary to reproduce SPS and SPSD responses, but is needed for resetting the system, in order to account for the fact that it does not show SPS and SPSD responses a long time after the last dry stimulation.

The most critical assumption, namely, that the natural frequency ω_j is distributed almost continuously over a wide range, can be justified by the more realistic assumption that the actual frequency distribution consists of several discrete major frequencies with deviations that overlap neighboring major frequencies. Each of the major frequencies corresponds to a mode of specific biochemical oscillation. This picture also explains why oscillators of the same frequency should be summed before summing those different frequencies.

Oscillators with the same biochemical identity can interact over a distance by chemical diffusion and active advection of protoplasmic streaming. These direct interactions will tend to synchronize phase, but it may not be enough to make a strong synchrony. In fact, macroscopic cellular behaviors often show fluctuating oscillatory variations rather than clear oscillations with a large amplitude, and the fluctuating oscillatory variations of different chemical identities display frequent switching between in-phase and out-of-phase relationships, although each biochemical component still shows the slightly oscillatory behavior. This consideration implies that the noise ξ should be small so that desynchronization effects are relatively weak.

The existence of the SPS response means that the organism anticipates the next periodic environmental change. To perform this function, it needs the ability to memorize the periodicity. Moreover, the organism memorizes not only the periodicity but also the specific phase of a period. Pursuing this line of reasoning, we might conclude that the *Physarum* plasmodium can perform a primitive version of brain function (that is, memory and anticipation). It should be noted that, in the case of the SPSD response, the single stimulation provides no information about periodicity. This means that the previous periodicity must be not only stored but also evoked by the trigger stimulation. An activity of this kind is known as recall. It is unclear, however, whether the plasmodium actually has the ability to recall, because the SPSD response was seen only at $\tau = 60$ min.

It is not common to encounter recurrent environmental changes with periods of 0.5–1.5 h in nature. Thus, some might consider the capacity to learn a periodicity of this kind to be meaningless. However, the opposite conclusion could also be drawn, namely, that the organism is able to remember periodic changes that it has not experienced before. This indicates that the organism has a generalized capacity for learning, independent of the details of the periodicity.

Discerning a periodicity is not easy, even for humans. According to history textbooks, when the ancient Egyptians recognized the regular periodicity of the flooding of the river Nile and succeeded in anticipating the next flood, this breakthrough triggered the invention of the calendar and was a symbol of the dawn of civilization. It is thus remarkable that a single-celled organism can perform such a function.

Although the results described in this Letter were obtained in the true slime mold, more primitive organisms, such as bacteria, can demonstrate intelligent behavior with a simple mechanism in terms of nonlinear dynamics [16]. The nonlinear dynamics perspective might thus be the key to revealing the secret of how biological systems overcome challenges to their survival.

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