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Amoebae Anticipate Periodic Events

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When plasmodia of the true slime mold Physarum polycephalum 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 cm²) 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 cm²) 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 (τ = 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.
or longer than 90 min were not tested due to technical
similar for all periods. Time periods shorter than 30 min
approximately 40%–50%, and this figure was roughly
all the values of
failed to undergo SPS. Instances of SPS were evident for
occurrences of SPS under these conditions. At
and
C
served. However, as shown in Fig. 1, SPS reappeared at
locomotion speed fluctuated and no further SPS was ob-


S
SPS after one disappearance (SPSD). SPSD was
Single dry stimulation at
C
served. The SPS response was thus de-
stimulation, and those below 5 mg wet weight could be
of 5–10 mg wet weight, were more sensitive to the dry
Fig. 2 were 10–19 mg. The smaller plasmodia, in the range


S
SPS: black denotes three, dark gray denotes two, light gray
denotes one, and white denotes none. The twofold criteria for
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FIG. 1. SPS and SPSD responses. Typical time course of loco-
motion for an organism (wet weight = 15 mg) before and after
three periodic applications (S1, S2, and S3) and single applica-
tion (S4) of dry stimulation. (a) Temperature is denoted by the
upper line (TM) and humidity by the lower line (HU). (b) Loco-
motion 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).


After a few occurrences of SPS at τ = 60 min, the
locomotion speed fluctuated and no further SPS was ob-


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. 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.


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 locomo-
tion speed between the last stimulation and the time of the
subsequent virtual stimulation, and that the minimum locomo-
tion 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 60% 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 oscillators exist. These oscillators are a series of oscillations underlie the multirhythmicity of locomotion, as multiple rhythms were observed in cellular activities in a Physarum plasmodium [6,7]. These cell movements showed a (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 \( \frac{d\theta_{ij}}{dt} = \omega_j (0 \leq \theta < 1) \), where \( \theta_{ij} \) is the phase of an oscillator of a frequency \( \omega_j \). 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 \( \omega_j \). For this oscillator group to respond to the external periodic dry stimulation, the dynamics can be written as

\[
\frac{d\theta_{ij}}{dt} = \omega_j + \alpha H(t) \sin(2\pi\theta_{ij}) + \xi_{ij},
\]

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 \( \omega_{ext} \) 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

\[
S = \sum_i \tanh \left( 2 \sum_j \cos \frac{2\pi\theta_{ij}}{N} + 3 \right).
\]

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 oscillators is different as some coherent groups of oscillators persist, as shown in Fig. 4(b). 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.
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 $\omega_j$ is distributed almost continuously over a
wide range, can be justified by the more realistic assump-
tion that the actual frequency distribution consists of sev-
eral discrete major frequencies with deviations that overlap
neighboring major frequencies. Each of the major frequen-
cies corresponds to a mode of specific biochemical oscil-
lation. 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 in-
teract over a distance by chemical diffusion and active
advection of protoplasmic streaming. These direct inter-
dactions 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 varia-
tions 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 oscil-
latory behavior. This consideration implies that the noise $\xi$
should be small so that desynchronization effects are rela-
tively weak.

The existence of the SPS response means that the or-
ganism anticipates the next periodic environmental
change. To perform this function, it needs the ability to
memorize the periodicity. Moreover, the organism memo-
rizes 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 anticipa-
tion). 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 generaliza-
ted 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 flood-
ing 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 per-
form such a function.

Although the results described in this Letter were ob-
tained 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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