Diversity and Commonality in Animals

Shuichi Shigeno
Yasunori Murakami
Tadashi Nomura *Editors*

Brain Evolution by Design

From Neural Origin to Cognitive Architecture

Springer

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Preface

The present book is a new, detailed examination to explain how elegant brains have been shaped by simple principles in evolution. Classic comparative studies have revealed great diversity of neural networks and complex behaviors in many animal groups. The recent integrative molecular, developmental, physiological, and psychological approaches, however, have revealed unexpected commonality in the basic structures and functions across animal phylogeny. The structural frameworks of the nervous systems and brains are often replicated in artificial intelligence or machines constructed by human activities, suggesting that functional similarities provide a common design for information processing systems through biophysical constraints.

The book introduces the origin of neurons with the single-cell creatures without neurons and then goes on to primordial types in invertebrates such as cnidarians, flatworms, molluscs, insects, and chordates, with a great abundance of the brains of vertebrates: fish, reptiles, birds, and mammals, including whales and humans. Recently, a number of research investigations of diverse and minor organisms have been conducted, and we need to keep up to date. Each chapter provides professional and detailed topics about brain evolution; however, this book as a whole is arranged along a simple concept to find something of common design. Also, non-organisms such as models and materials are covered to explore the designs in the origin and evolutionary processes, but they are not comprehensive. The topics are provided in a timely manner because novel techniques emerged rapidly, for example, as seen in next-generation sequencers and omics (e.g., genomics, proteomics, metabolomics, and connectomics) approaches. With the explosion of big data, the neural-related genes and molecules are now on the radar.

Importantly, now the neural networks have been taken notice of. For instance, Europe's €1 billion science and technology projects, such as the Human Brain Project, were launched in 2013 to analyze brain connectomics. The big interdisciplinary plan, the Blue Brain Project, also aims to understand the small mammalian brain. Furthermore, with the rise of recently advanced artificial neural networks, there is enthusiasm for the development of neural network models. The views of brain evolution provide an essential opportunity to generate ideas for novel neuron-

and brain-inspired computation. For that reason, this book will show the reader how to extract meaningful neural structures in nature.

For undergraduates, graduate students, and professional scientists who seek a deeper understanding, this volume demonstrates how to find the basic principles shaping brains that provided higher cognitive functions in the course of evolution. Our ambition is that the book will stimulate students, particularly young scientists, to delve into problems remaining in this discipline. Many authors were selected from young Japanese scientists, and this work is part of a series of publications of the Zoological Society of Japan.

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Part I
The Origins of Neurons and Networks

Chapter 1
Physical Ethology of Unicellular Organisms

Shigeru Kuroda, Seiji Takagi, Tetsu Saigusa, and Toshiyuki Nakagaki

Abstract In this chapter, some behaviours of unicellular organisms that appear to be smart or intelligent are reported. Two topics are the focus from two major groups of eukaryotic unicellular organisms, amoebae and ciliates: (1) anticipatory capacity of periodic environmental events in an amoeba and (2) environment-induced development of a new type of behaviour in a ciliate. A mechanism of these behaviours is discussed, based on a mechanical equation of motion. Ethology (the science of animal behaviour) of unicellular organisms is recently being studied from a physical point of view. We propose to call this kind of study {physical ethology}. Physical ethology may give us some hints about the origin of primitive intelligence.

Keywords Ciliate • Learning • Adaptability • Primitive intelligence • Membrane excitability • Ethology • Nonlinear dynamics

1.1 Introduction

The cell is the building block of all organisms, which can be the minimum set of life, because the cell is not alive if it is divided into subsystems: this implies that all essence to be common throughout the entire range of organisms must be found in a cell. So, the cell is the interface between an assembly of merely materials and the functional states of living systems.

In fact, many kinds of smart behaviour that look like a primitive form of intelligence in some sense have been reported and compared with so-called intelligent behaviours in higher animals (Jennings 1906; Bray 2009; Eisenstein 1975; Corning and Von Burg 1973; Trewavas 2003, 2005).

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Although the list of such behaviours is getting longer and longer, the mechanism of the smartness is being studied from a mechanical point of view. This is an attempt to understand an origin of organismic smartness in nature and is an exciting problem to be tackled by truly interdisciplinary science in this century. Here, we wish to shed light on the attempt.

Two of the impressive examples are maze solving (Nakagaki et al. 2000, 2001; Tero et al. 2006, 2007) and anticipating periodic events (Saigusa et al. 2008) by amoebae. *Physarum*, which recently has become a focus as an interesting model organism, is a huge amoeba that constructs a transport network of information and materials and mimics an anthropogenic network of public transportation. This description implies that there may be a common nature of network formation shared by man and *Physarum* (Tero et al. 2010).

The other example of smart behaviour is the environment-induced development of new types of behaviour in a ciliate (Kunita et al. 2014). In the behaviour of a ciliate, swimming activity is regulated by membrane potential (Naitoh 1990), and an equation of motion for the membrane potential is described by the reaction–diffusion equation of the Hodgkin–Huxley type, which was originally proposed for the squid neuron. Because of the similarity of excitability of membrane potential, the ciliate is often studied as a model system of neuronal activity (Eisenstein 1975; Naitoh 1990).

So far, the attempts to seek for a physical origin of smart behaviours are just at the beginning, but this line of study, which we propose to name physical ethology, is exciting and promising for the future.

In this chapter, as an introduction to the physical ethology of a cell, we describe in more detail one of the symbolic topics rather than the original report already studied: "*Physarum* anticipates periodic events" (Saigusa et al. 2008). Although a mathematical model for anticipatory behaviour was proposed and discussed, we do not repeat it here. [Please see the reference (Saigusa et al. 2008) for explanation of the model, which supplies the main body of this chapter.]

After that, we briefly mention one more topic of the swimming behaviour of ciliates because the cilia are the other main character of Protozoa as well as the amoebas. A new kind of smart behaviour in ciliates, reported recently in 2014 (Kunita et al. 2014), is described in short as a summary. The mechanism is reduced to the dynamics of membrane potential that controls the beating activity of the cilia. So, this is another typical example of physical ethology.

At the end of this chapter, some concluding remarks are given on the possible origin of intelligent behaviour that has emerged at the level of a single cell.

1.2 Anticipatory and Recall Behaviour in Response to Periodic Stimulation in the Plasmodia of *Physarum polycephalum*

1.2.1 Overview and Background

We report on adaptive responses to periodic stimulation in *Physarum polycephalum* (Mycetozoa), which exhibits primitive anticipatory and recall behaviour. The behaviour studied here involved the spontaneous periodic slowdown of migration just after a series of periodic stimulations had been experienced (anticipatory behaviour). This slowdown subsequently disappeared but then reappeared after a single stimulation (recall behaviour). The behaviour displayed was characterized by the following features. (1) The anticipatory slowdown depended on the size of the body. (2) The response varied across different parts of the body. (3) Recall behaviour appeared even in organisms that had failed to display anticipation earlier. (4) The cellular rhythm of the contraction movement was much slower during both the spontaneous slowdown (anticipation) and recall behaviour than during free locomotion; the same response was shown during the slowdown directly induced by external stimulation. The results obtained here give new insight about how this ability of anticipation and recall emerges at the cell level.

The repetition of a particular event often leads to the formation of memory and learning in organisms. For example, after mice were fed several times at regular intervals, they learned to anticipate the next feeding time (Roberts and Church 1978; Church 1978; Meck et al. 1984). When bees were given nectar at 9:00 a.m. every day, they gathered at the scheduled time even if nectar was not given (Gould and Gold 1988). These are elegant examples of anticipatory behaviour towards periodic events.

In 2008 we reported that such behaviour can be observed even in a single cell, despite the absence of any brain or nervous system (Saigusa et al. 2008). Similar behaviours could be observed in other protozoa (*Brepharisma*) and a plant (*Chara*) (Kunita et al. 2013), but the anticipatory behaviours in these species were not so clear as statistical fluctuations were large and the organisms often failed the anticipation. Thus, further characterization is needed as this finding is interesting from an evolutionary point of view and gives a hint as to the cellular origin of primitive intelligence (Ball 2008).

The anticipatory behaviour reported in the paper by (Saigusa et al. 2008) is as follows. The plasmodium of the true slime mould *Physarum polycephalum* moves rapidly under favourable conditions, but stops moving when experiencing less favourable conditions. Plasmodia exposed to unfavourable conditions (low temperature and low humidity), presented in three consecutive pulses at constant intervals, were found to reduce their locomotive speed in response to each episode. When subsequently subjected to favourable conditions, the plasmodia spontaneously reduced their locomotive speed at the point in time when the next unfavourable episode would have occurred. This finding implies that the plasmodia can anticipate impending environmental change. After this behaviour had been evoked several

times in the course of favourable conditions, the locomotion of the plasmodia returned to normal; however, the anticipatory response could subsequently be induced again by a single unfavourable pulse, implying recall of the memorized periodicity.

To focus on deviation and fluctuation of response, we added new experiments and analysed the deviation by reexamining a large set of data that included not only the new data but also the previous data (Saigusa et al. 2008); this is the first point to be considered here.

The second point is dependency on the internal conditions of the cell. In general, behavioural responses to environmental conditions depend on the internal conditions of the cell, but little is known about it. So, we will test the possible factors of cellular conditions: size of body, spatial inhomogeneity in parts of a large body, and effects on rhythmic contraction of the cell.

We study further details of the anticipatory behaviour and report several new findings and insights.

1.2.2 Materials and Methods

Organisms and Culture The plasmodium of *Physarum polycephalum* (wild type) was cultured using oat flakes on an agar plate in a rectangular plastic dish (25×35 cm^2) at 26 °C and humidity of 90 % according to conventional methods.

Observation of Locomotion Velocity Under Periodic Changes in Atmospheric Temperature and Humidity The tip portion of the large cultured organism was cut out and placed at the end of a narrow rectangular area (0.5×28 cm) on the agar plate, hereafter referred as an arena, as shown in Fig. 1.1a. The wet weight of the plasmodial mass that was initially placed in the arena varied from 5 to 40 mg, depending on the particular experiment. The arena containing the specimen was kept in the dark in an incubator (KCL-10000; Eyela) in which the atmospheric humidity and temperature were controllable. The migrating organism was illuminated from below by a matrix of infrared LEDs and viewed by a CCD camera. Under the culture conditions used, the plasmodium started to move towards the other end of the arena approximately half an hour later and was allowed to move freely for 4 h. The atmospheric temperature and humidity were then changed to 23 °C and 60 % for 10 min, conditions that represent the stimulation. This stimulation was repeated three times at intervals of τ ($\tau = 30, 40, 50, 60, 70, 80, 90$ min). The position of the extending front of the migrating plasmodium was measured every 10 min; typical time courses of the tip position are shown in Fig. 1.1b.

Typical Response Analysed in This Study, Originally Reported in 2008 (Saigusa et al. 2008) A typical time course of speed and acceleration of migration as well as the time course of temperature and humidity are shown in Fig. 1.1c, as reported in the previous papers. According to the terminology used in the previous paper, the periodic stimulations were numbered from S1 to S2 and S3 (indicated by gray bars),

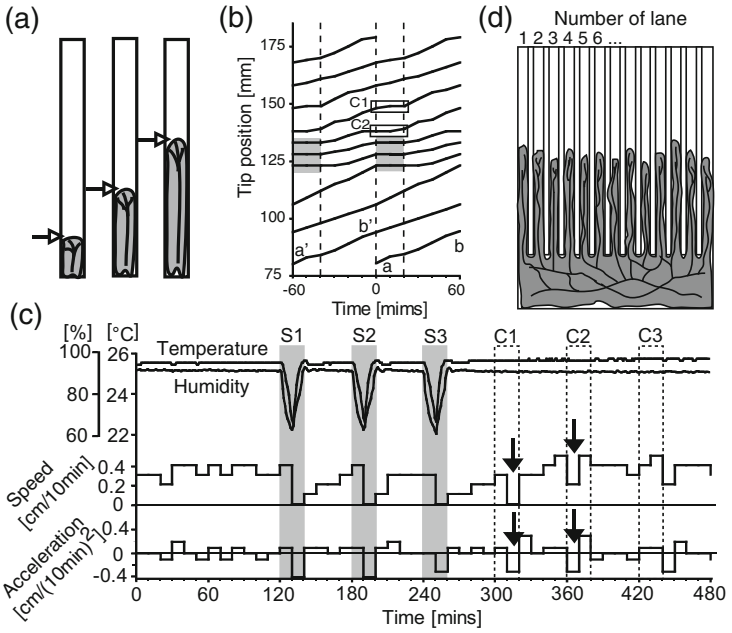

Fig. 1.1 Schematic illustration of experimental setups used to study *Physarum*. (**a**) Illustration of migration along a narrow lane from bottom to top. *Arrow* indicates the frontal tip of the organism, the position of which was used to calculate locomotion speed. Arena size was 0.5×28 cm^2. (**b**) Typical plot of tip position with respect to time. The plot has been cut into sections of 60 min that are stacked vertically; each section includes the preceding 60 min (plotted as negative time) to help recognize periodic responses. For instance, the line *a–b* is identical to a'–b'. The column colored *grey* indicates the times at which stimulation occurred. (**c**) Typical time course for locomotion speed in response to periodic stimulation. *Black arrows* indicate spontaneous slowdown (SPS) at times when the next stimulation would have occurred if continued periodically. The wet weight of the initially inoculated organism was 15 mg. Speed and acceleration were averaged every 10 min. Sn and Cn were timing of actual stimulation and the following periodic timing (no stimulation). This response was originally reported in the previous paper (Saigusa et al. 2008). (**d**) Setup for the 'comb' experiment in which the arena was shaped like a comb with 12 teeth into which the plasmodium could extend a pseudopod-like protrusion. The migration speed was measured along every tooth. Tooth size was 0.5×25 cm^2

and the following periodic points in time (no stimulation was applied) were C1, C2, C3, and so on (indicated by dashed rectangles). In C1 and C2 only, the locomotion speed often decreased spontaneously, so this response was called spontaneous in-phase slowdown (SPS) hereafter.

An Arena with Comb-like Shape Designed for Testing Inhomogeneity of Response Over the Whole Body Another arena in the form of a comb that consisted of many parallel slender corridors with a common base was prepared (Fig. 1.1d). The large plasmodium was placed in the base and started to extend along the many corridors while remaining as a single entity.

Spatiotemporal Observation for Rhythmic Contraction According to the conventional method (Takagi and Ueda 2008), we measured contractile activity in the plasmodium. The plasmodium was illuminated by the 20×20 matrix of infrared light emission diode (wave length, 920 nm) from below, and was viewed from above by the black-and-white CCD camera (spatial resolution, 480×640; time resolution, 1/30 s). As the gray level of the video image reflected the thickness of the plasmodium that changed periodically with accompanying to active rhythmic contraction, a darker pixel indicated a thicker part of plasmodium. The relationship between optical density on the image and the real thickness of organism was standardized by the calibration. We used the standard free software for image analysis Image J that was prepared by the National Institutes of Health (USA). A spatiotemporal pattern of oscillation periods was visualized as shown in Fig. 1.6.

1.2.3 Results

Effect of Body Size on Anticipatory Behaviour Figure 1.2 shows averaged time courses of the migration speed. The plasmodia that were initially inoculated varied in wet weight, and the difference in body size was classified into three groups: 5–9 mg ($n = 27$), 10–19 mg ($n = 68$), and 20–40 mg ($n = 21$). The initial speed before stimulation was similar for all three groups but differed after stimulation. Although the group with large body size recovered its previous migration activity after stimulation, the groups of medium and small body size did not move as fast as before stimulation. This finding implies that stimulation caused serious damage to the smaller organisms. In the group with body size from 5 to 9 mg wet weight,

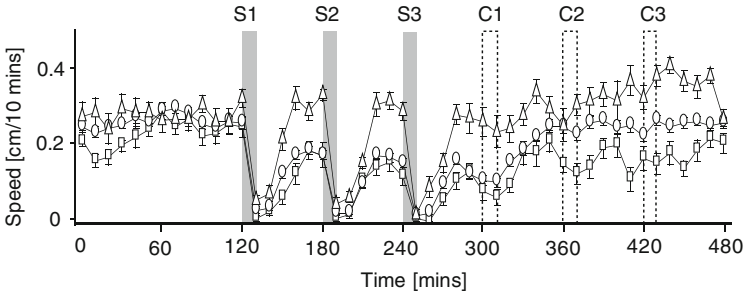

Fig. 1.2 Confirmation of anticipatory behaviour in *Physarum* obtained by measuring averaged speed of locomotion. Different body masses were initially inoculated for the three time courses shown: small (5–9 mg, $n = 27$, *lower line* with *rectangular* symbols), medium (10–9 mg, $n = 68$, *middle line* with *circular symbols*), and large (20–56 mg, $n = 21$, *upper line* with *triangular symbols*). Anticipatory behaviour is observed at C1 and C2. Mean ± standard error of mean. Statistical significance of difference was tested by pairwise *t* test with Bonferroni correction (a method of multiple comparison). The *p* value was less than 0.001 between the small and the large, and between the medium and the large, at the time points of C1 and C2, and between all pairs at C3

some plasmodia did not migrate at all after the first stimulation (data not shown). Such cases were excluded from the results shown in Fig. 1.2 and 21 experiments when finally averaged. When stronger stimulation was applied (for example, 18 °C and 50 % humidity), even larger plasmodia were damaged by a single stimulation.

The spontaneous slowdown response that was observed depended on a balance between body size and the magnitude of the environmental perturbations. Anticipatory behaviour was shown in all three groups at the times of the fourth (C1) and fifth (C2) virtual stimulations. The anticipatory response was clearer for smaller plasmodia because the ratio of the migration speeds in the two phases of slowdown and recovery was higher than for larger plasmodia. The smaller organisms demonstrated clearer anticipation but suffered more serious damage. The anticipation capacity thus depends on both internal and external conditions.

Response in Local Parts of the Body Figure 1.3 shows time courses of the migration acceleration for the comb-shaped plasmodium (see Materials and Methods for details). The lane numbers indicate different parts of the body corresponding to different 'teeth' of the comb-shaped arena. After stimulation had been applied at times S1, S2, and S3, anticipatory behaviour was expected at times C1, C2, and C3. Anticipatory behaviour was indeed observed as the overall tendency (black squares indicate deceleration). However, the expected response was not always observed in individual lanes. For example, it was absent in lanes 1–3, 7, and 10 at C1, and present in lanes, 6, 8, 9, and 11. The varying behaviour between different local parts of the body implies that the anticipatory response is subject to fluctuations and uncertainty. This comb-type experiment was repeated three times, and similar inhomogeneity of response was observed in local parts of the body.

Response to Different Periods of Stimulation Figure 1.4 shows the occurrence of spontaneous slowdown in response to various periods of stimulation in the range 30–90 min. In all cases, the periodic stimulation was applied three times (S1, S2, and S3) and the time axis was set to zero where the last stimulation (S3) was given. The first, second, and third occasions of virtual stimulation are indicated by C1, C2, and C3, respectively. At C1, the statistical occurrence of slowdown was high (10 %–50 %) for all periods of stimulation. The baseline occurrence of slowdown in the absence of stimulation was approximately 10 %. At C2, a high occurrence of slowdown

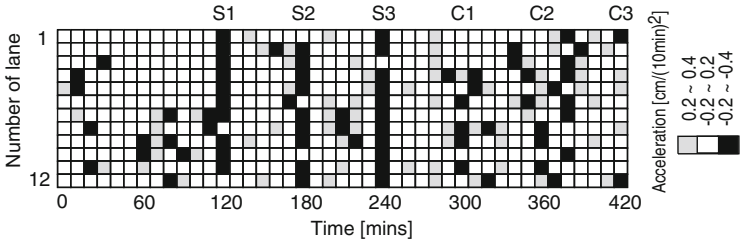

Fig. 1.3 Acceleration as a function of time for different lanes of the comb-shaped arena. The *grey squares* indicate positive acceleration and the *dark squares* indicate braking

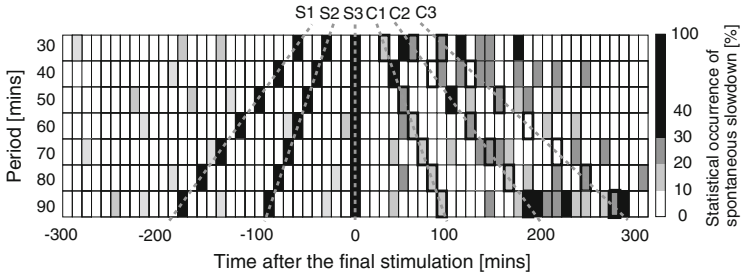

Fig. 1.4 Anticipatory response for stimulation periods ranging from 30 min to 90 min. The *grey scale* indicates the statistical occurrence of spontaneous slowdown. Zero time was defined as the time when the last stimulation S3 was applied. Spontaneous slowdown was defined to occur when acceleration ≤ -0.2 mm/(10 min). The numbers of repeated experiments for each stimulation period were 36 (30 min), 46 (40 min), 34 (50 min), 121 (60 min), 40 (70 min), 67 (80 min), and 39 (90 min). The wet weight of the organism was 10–19 mg. Data that were already used in the previous paper (Saigusa et al. 2008) were included in this analysis while we supplemented new data of 97 repeats to make this new analysis of fluctuation sufficiently reliable. We analyzed all the data, not only the previous ones but also the new data

was still observed although the timing of the response was sometimes shifted a little earlier or later. At C3, the anticipatory response was no longer significant as the timing greatly fluctuated. In each experiment for a given stimulation period, there was a degree of deviation in the time at which spontaneous slowdown was observed. The anticipatory responses were often advanced or delayed by as much as a quarter of a period, which can be seen as a characteristic of this type of behaviour. Nevertheless, there is a clear tendency towards a high probability of occurrence along line C1 in Fig. 1.4. We conclude that anticipatory spontaneous slowdown occurred for a wide range of periods from 30 min to 90 min.

Recall Behaviour With and Without Anticipatory Behaviour Figure 1.5 shows time courses of slowdown occurrence and averaged speed in response to a single stimulation that was applied 5 h after the series of three periodic stimulations. After the anticipatory response at C1, the locomotion speed quickly returned to the value that it had before the sequence of stimulations. A further single stimulation was then given at S4. In response, spontaneous slowdown was displayed at C6, which corresponds to the period of stimulation previously applied for S1–S3 (1 h). This response implies that the organism had stored a memory of the periodicity somewhere in its body that was later retrieved. This type of behaviour can be seen as a primitive form of recall. The white bars and circles in Fig. 1.5 indicate the responses of a group of organisms that showed anticipatory behaviour at C1, whereas the black bars and circles represent the group that failed to display this behaviour. Recall behaviour was observed even for the group that failed anticipatory behaviour, although the response was slightly weaker than that of the successful group. Thus, recall was not restricted to individuals that succeeded in showing anticipatory behaviour, which implies that storing a memory is a process that occurs

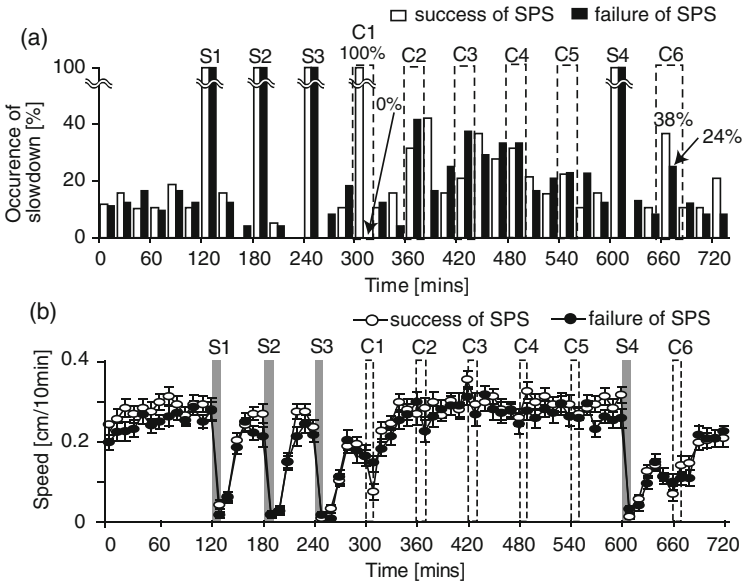

Fig. 1.5 Recall behaviour in response to stimulation S4 applied after the anticipatory response has disappeared, studied by measuring the statistical occurrence of slowdown (**a**) and averaged speed (**b**) over 40 repeats. The criterion for spontaneous slowdown was acceleration ≤ -0.2 mm/(10 min). The wet weight of the organism was 15 mg. The data of the success of SPS shown in Fig. 1.5b were the same as those previously published (Saigusa et al. 2008). The number of repeats was 40, and this number of repeats was the sum of the new data and the previous experiments (Saigusa et al. 2008). Mean $\pm$ standard error of mean. Statistical significance of difference was tested by *t* test. The *p* value was not observed to be less than 0.01 between the success and the failure groups at the time points of C1, C2–C5, and C6

in the body of both groups. Despite fluctuations in the times at which anticipation and recall took place, it is clear that *Physarum* is able to store meaningful information in the form of time memory.

Modulation of Rhythmic Contraction During Spontaneous Slowdown Figure 1.6 shows a typical time course of the rhythmic contraction that occurred when the organism showed anticipatory behaviour. The plasmodium usually displays periodic cycles of contraction and relaxation over the entire body with an average period τ of approximately 1 min, as shown by the spatiotemporal plot of the oscillation period in Fig. 1.6a. The oscillation period of the entire body became 70 s longer when stimulation was applied at S1, S2, and S3. During the spontaneous slowdown of locomotion speed at C1, slower oscillations were observed (τ 90 s), but this increase in period was smaller than that at S1, S2, and S3. Figure 1.6b, c shows the time courses of the oscillation period, averaged over the entire body, and of the locomotion speed, respectively. An increase in the period of the contraction rhythm was clearly observed at C1 in Fig. 1.6b. A minimum in the time course of the speed was also observed at C1 (Fig. 1.6c), as occurred at S1, S2, and S3. That is,

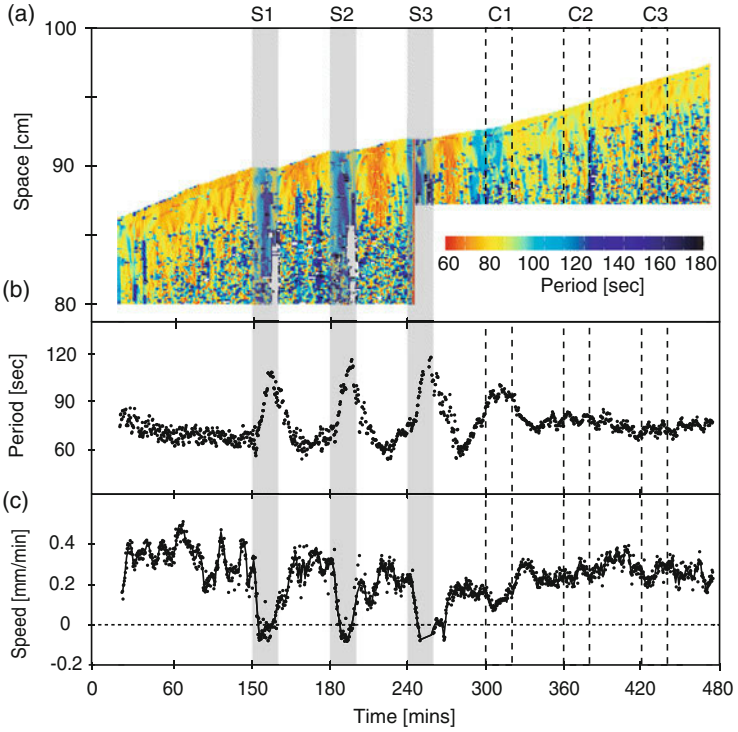

Fig. 1.6 Modulation of cellular rhythm in response to periodic stimulation. (**a**) Period of rhythmic contraction as a function of time. (**b**) Time course of contraction period, averaged over frontal part of organism (1 cm from extending tip). (**c**) Speed of locomotion with respect to time: a typical response in all three repeats. Body weight initially inoculated was 10–19 mg

the rhythmic contraction slowed down whenever the locomotion speed decreased, whether induced by external stimulation or as a result of spontaneous anticipatory behaviour. This result implies that the elementary process of anticipatory response involves not only the overall behaviour of locomotion but also the local kinetics of biochemical oscillatory reactions. Figure 1.7 shows the changes in oscillation period (Fig. 1.7a) and locomotion speed (Fig. 1.7b) associated with the recall behaviour at C6 (the experimental conditions were the same as in Fig. 1.5.). In the time course of the contraction period, a maximum similar to that observed for the anticipatory response was displayed at C6. The time course of the locomotion speed showed a maximum between S4 and C6 and a minimum at the beginning of C6. That is, a slowdown in both contraction rhythm (maximum of period) and locomotion (minimum of speed) occurred during the recall behaviour around C6. In this sense, the recall behaviour was similar to both the anticipatory behaviour and the slowdown induced by external stimulation.

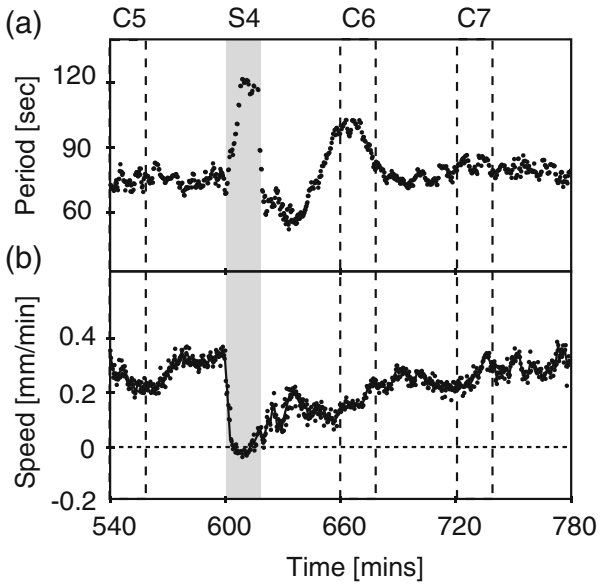

Fig. 1.7 Modulation of contraction rhythm during recall response. (**a**) Time course of contraction period after stimulation S4. (**b**) Time course of locomotion speed: a typical response in all three repeats. Body weight initially inoculated was 10–19 mg

1.2.4 Discussion

Further Examination of Data and Confirmation of Dependency of Applied frequency Although the previous paper shows just the findings of the anticipatory behaviour and, in particular, information on dependency of applied periodicity is very limited, the current paper shows a more consistent analysis of data, in which not only the new data but also the previous data of the experiment are included. Figure 1.4 gives an overall picture of anticipatory response in the parameter space that was spanned by the applied periodicity and time. The anticipatory response was observed to show a deviation of time to spontaneous slowdown around the correct time points C1 and C2, but the anticipatory response was clearly observed around C1 when we looked through a series of periodicity. The time deviation of SPS increased as the time progressed from C1 to C3. Around C3, the anticipatory response was no longer observed. When we consider justification for the physical mechanism proposed for the anticipatory behaviour, statistical distribution of the deviation may give us helpful information.

As shown in Fig. 1.5a, the plasmodia that failed the first anticipation (0 % at C1) were able to succeed in the second and third anticipation (approximately 30–40 % at C2 and C3). This is remarkable. It is interesting to check whether this response is reproduced by the previously proposed mathematical model.

Size Effect In general, it is known that the larger plasmodium is more resistive against sudden changes in environmental conditions such as temperature and humidity. For instance, although one large organism can survive against a drop in humidity although some part of the large body is necrotic, a much smaller one cannot survive against the same drop in humidity. This difference may be because loss of water vapour from the surface of the body is relatively higher in smaller organisms: the surface–volume ratio increases as the body size decreases, in principle. So, we expect that smaller organisms tend to be more sensitive to the periodic environmental change. If body size, however, is smaller than the critical size, the organism can die or try to transform to a resting stage such as the spore and sclerotium. The anticipatory behaviours observed here obey a balance between maintenance of vegetative stage (plasmodium) and morphological transformation to resting stage (spore and sclerotium). It is reasonable that a smaller plasmodium is sensitive to the periodic changes in humidity and temperature as facing to higher risk of survival.

Inhomogeneity of Response in a Whole Body We discuss the result that the anticipatory response was not homogeneous in the whole body but differed from part to part: one part might be successful while another part is not. According to the implication of the previously published model for the anticipatory response in *Physarum* (Saigusa et al. 2008), the capacity is based on the chemical kinetics of a complicated network of biochemical reactions. It is reasonable that biochemical reactions are very similar over the body but states of reaction do not always synchronize because chemical diffusion and protoplasmic streaming may not be always sufficient for maintaining perfect synchronization throughout the body. In fact, some chemicals such as ATP, Ca^{2+}, and NAD(P)H show inhomogeneous distribution in the body and moreover such inhomogeneity is actively related to the development of amoeboid behaviour in *Physarum* (Nakamura and Kamiya 1985; Ueda et al. 1990; Ueda 1993; Yoshiyama et al. 2010). So, spatial differences of anticipatory response may result from the inhomogeneity of dynamic states of reaction kinetics.

Recall with No Anticipation In the results shown in this paper, the plasmodium that failed the anticipatory response sometimes succeeded in the recall behaviour. According to the mathematical model previously proposed (Saigusa et al. 2008), the core process in kinetic motion of biochemical reactions in the plasmodium is that two kinds of phase synchronization take place: synchronization (or formation of phase cluster, in other words) between biochemical oscillators that have the same natural frequency (the same biochemical species, for instance), and synchronization between the these phase clusters that have a slightly different natural frequency. The second synchronization is related to recall behaviour whereas the first one is related to storing the memory of periodicity. As these two synchronization phenomena can be separable, we may expect that recall is possible without anticipation. It is interesting that this possibility could be examined in the conventional mathematical model for anticipatory and recall behaviours in the future.

The plasmodia that failed the first anticipation (0 % at C1; Fig. 1.5a) were able to succeed the second and third anticipation (approximately 30–40 % at C2 and C3). This is remarkable. It is interesting to check whether this response is reproduced by the previously proposed mathematical model.

Effects on Contraction Rhythms In this paper, information on intracellular states was shown for the first time: modulation of intracellular rhythm. The contraction rhythm (the typical period is 1–2 min) was modulated in response to the environmental changes and the same modulation was confirmed in the spontaneous slowdown of anticipatory and recall behaviours. Because this rhythmicity was much faster than we had considered in the mathematical modelling (applied periods were 30–90 min) in the previous paper (Saigusa et al. 2008), the information from this result does not help to examine the model justification directly. At least, we can extract the message that oscillatory activity in the plasmodium was modulated in relationship to development of anticipatory and recall behaviours. Further observation and examination of frequency modulation at a slower time scale is interesting in future studies to clarify the physical mechanism of the anticipatory and recall behaviour at cellular level.

Possible Approach to Physical Mechanism We have confirmed the previous finding (Saigusa et al. 2008) by reexamining not only new data but also the previous data together. *Physarum* exhibits anticipatory and recall behaviour in response to periodic changes in environmental conditions. Humans have a similar capacity. Our study implies that this type of capacity is not specific to a single species but rather may be found in multiple species distributed over a wide range of the phylogenetic tree. Therefore, the underlying mechanism should be considered as a common process. One approach to studying such a mechanism is to search for specific genes and proteins that are involved, but an alternative approach is the modelling of physical dynamics. According to the latter, a simple and general model can be proposed: anticipatory and recall behaviour is reproduced by the collective motion of independent oscillators with a wide range of natural frequencies. This is a possible model for the anticipatory behaviour, but another set of model equations is also proposed (Tachikawa 2010). Although the discussions on the possible physical mechanism are still ongoing, it is remarkable that higher capacities such as anticipation and recall are created by the simple and general model of differential equations. The study of how protozoa anticipate periodic events hints at the origin of time memory from an evolutionary point of view.

Evaluation Method for Memory Capacity for Complexity of Time Sequence In this report, the stimulation was applied in regularly periodic fashion. The time sequence of the stimulation could be made more complex. For instance, possible sequences are (1) alternative changes in the stimulation magnitude, strong and weak, over a regular period; (2) double-frequency stimulations such as alternating long and short stimulation periods; and (3) a chaotic sequence of stimulations. According to the ideas presented here, we may be able to design a standard test to assess the capacity of time memory throughout the phylogenetic tree that will be performed in the future.

1.3 Electric Control of Behaviour in *Paramecium*

Paramecium is a genus of popular unicellular ciliated protozoa that you can find easily in the pond near your office. Their average length and width are about 200 μm and 60 μm, respectively. Although they have a simpler body plan than ours, their behaviours are so rich that we can be glued to the microscope for long moments. They have many cilia (about 10,000) on their body surfaces, and they swim by ciliary beating that is spatiotemporally coordinated through the entire body so that they form a metachronal wave.

They can change their swimming directions by changing the directions of the effective stroke of ciliary beating. Ordinarily, in a uniform environment, they swim forwards. However, when they encounter an obstacle, they swim backwards for a while by reversing the direction of the effective stroke of ciliary beating (reversal of ciliary beating), and after swinging their anterior end, they begin to swim forward in a new direction. This behaviour is called avoiding reaction (Jennings 1906).

The relationships between the behaviour and electrophysiological property of *Paramecium* has been intensively studied for more than 50 years. During normal forward swimming, they have an internally negative resting potential that is provided by the intracellular high K^+ and low Ca^{2+} concentrations. It was known that the increase in intraciliary concentration of free calcium ion ($[Ca^{2+}]_i$) is necessary for the reversal of ciliary beating, and it is produced by depolarization of the cellular membrane (Naitoh and Eckert 1969; Naitoh and Kaneko 1972).

The appearance of the avoiding reaction behaviour is explained as follows: When the organism collides a obstacle at the front end, the mechanosensitive Ca^{2+} channel opens. The resulting depolarization that occurred around the receptors spreads immediately to the entire body including cilia. The depolarization of the ciliary membrane opens the voltage-sensitive Ca^{2+} channels on the membrane so that Ca^{2+} flows into the cilia from the outside of the body. The increase of $[Ca^{2+}]_i$ provides the reversal of ciliary beating so that the organism begins to swim backwards. The ciliary reversal is continued during $[Ca^{2+}]_i$ over a certain critical concentration (Naitoh and Kaneko 1972). The relaxation of the $[Ca^{2+}]_i$ leads it to resume the forward swimming. These data shows that an excitable cellular membrane in the protozoan acts as a receptor of the external stimulus and a transmitter between the receptor and the effector, whereas those are enacted by neural systems in eumetazoans, including human beings (Fig. 1.8).

What kinds of adaptability can be shown by *Paramecium*? If they exist, we can expect to understand them from the point of view of the membrane potential phenomena and their succeeding biochemical reaction. Actually, various behaviours in Protozoa have been reported since 100 years ago (Jennings 1906; Smith 1908; Bramstedt 1935; Applewhilte 1979). Smith (Smith 1908) found a novel behaviour of *Paramecium* in a dead-ended capillary tube. In this experiment, the organism was put into a narrow tube of which the width was smaller than its length, enough not to be able to turn by the avoiding reaction. In the beginning, the organism swims forward towards the closed end. After colliding with the tube end at its front part,

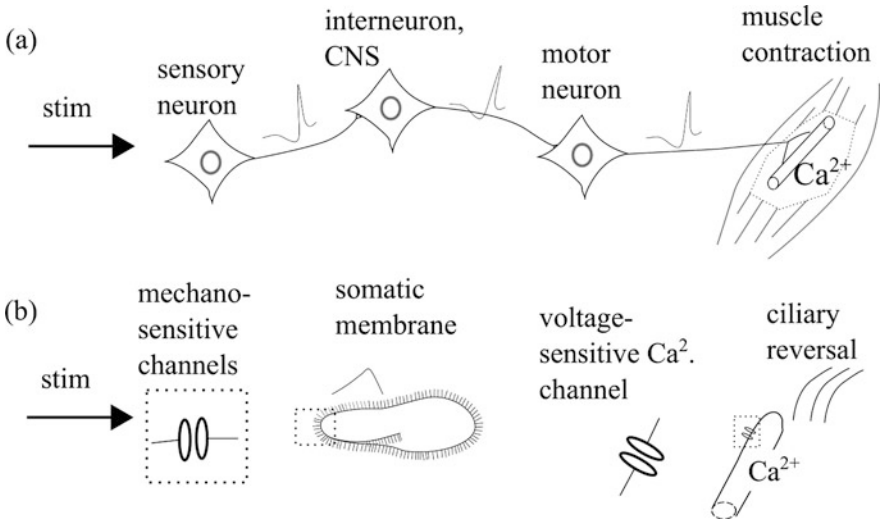

Fig. 1.8 Signal transmission by electrogenesis in (1) Eumetazoa and (2) Protozoa

it swims backward about its own length and resumes swimming forwardly; this is a part of the ordinary avoidance reaction and was repeated at least a dozen times. Finally, after some struggling, the organism succeeded to change its body direction by bending the body into a U-shape.

How do they behave when they encounter a more difficult situation? Recently we reported another novel behavior of *Paramecium* in a dead-ended capillary tube (Kunita et al. 2014). We used a narrower tube so that *Paramecium* cannot change its body direction by bending its body. The typical behaviour is as follows: After the organism was put into the tube, as in Smith's experiment, the ordinary avoiding reaction appeared at the closed end. That is, the organism approached a forward end and repeated back-and-forth swimming with a short distance (<0.5 mm). After that, for about 1 min, the distance of backward swimming gradually increased and finally reached a maximum distance (3–4 mm). It continued the long-distance backward swimming for a few minutes. We called the emergent long-distance backward swimming 'long-term backward swimming (LBS),' whereas that in the ordinary avoiding reaction is short-term backward swimming (SBS).

1.3.1 Paramecium Model

What is a underlying mechanism of the emergence of LBS? Naitoh (Naitoh 1990) has reported that a long-term application of outward current to the paramecium provided long-lasting backward beating of cilia even after the early inward Ca^{2+} current disappeared. Naitoh suggested the long-lasting backward swimming is

caused by a small-amplitude long-lasting inward Ca^{2+} current that flows into the same Ca^{2+} channel for the early inward Ca^{2+} current. Based on such previous studies, we exploited a *Paramecium* model using a conductance-based model and attempt to demonstrate the LBS.

Ciliary Membrane Let us begin with modelling of the ciliary membrane based on a conductance-based model. The total ciliary membrane current (I_M) is the sum of the capacitive (I_c) and the ionic currents (I_i), and so:

$$I_M = I_{\mathrm{c}} + I_{\mathrm{i}} = C_m \frac{dE}{dt} + I_{\mathrm{i}}, \tag{1.1}$$

where E is the potential difference between the between the outside and the inside of the membrane and the outward current is defined as positive current. The resting equilibrium potential ($\mathrm{E_r}$) is realized during ordinary forward swimming. For simplicity of notation, we use $\mathrm{V = E - E_r}$ instead of E in the following. It is known that the voltage-sensitive Ca^{2+} channel and also the voltage- sensitive K^+ channel are located primarily in the ciliary membrane whereas they are not found in the somatic membrane (Dunlap 1977; Machemer and Ogura 1979; Eckert and Brehm 1979). Then, the ionic current $\mathrm{I_i}$ is given by

$$I_i = I_{\mathrm{Ca}} + I_{\mathrm{K}} + I_{\mathrm{L}} \tag{1.2}$$

$$= g_{\mathrm{Ca}}(V,t)(V - V_{\mathrm{Ca}}) + g_{\mathrm{K}}(V,t)(V - V_{\mathrm{K}}) + \overline{g}_{\mathrm{L}}(V - V_{\mathrm{L}}), \tag{1.3}$$

where I_{Ca} and I_K are Ca^{2+} and K^+ currents, respectively; I_L is a relatively small voltage-independent conductance of undetermined ions; and V_{Ca}, V_K, and V_L are the equilibrium potential for the respective ions. We use the following conductance formula, which was proposed by Naitoh (Naitoh 1979; Naitoh and Sugino 1984).

$$g_{\mathrm{Ca}}(V,t) = \overline{g}_{\mathrm{Ca}} m(V,t)^5 \left(1 - (1 - h(V,t))^5\right), \tag{1.4}$$

$$g_{\mathrm{K}}(V,t) = \overline{g}_{\mathrm{K}} n(V,t). \tag{1.5}$$

Here, each gate variable m, h, or $n \in [0, 1]$ develops by $dz/dt = \alpha_z(V)(1 - z) - \beta_z(V)z$, where $\alpha_z(V)$, $\beta_z(V)$ are the pair of voltage-dependent reaction rates ($z = m, h, n$) that were determined by reference to the measurement data available from (Hirano et al. 2005).[1] In the following, time, potential, current density, conductance,

[1]The expression of the functions for the voltage-dependent reaction rates (α, β) were determined by reference to the measurement data available from (Hirano et al. 2005) as follows: $\alpha_{\mathrm{m}}(V) = 0.39(46.40 - V)/(\exp(0.039(46.40 - V)) - 1)$, $\beta_m(V) = 0.65 \exp(-V/15)$, $\alpha_h(V) = 0.05 \exp(-V/50)$, $\beta_h(V) = 1.0(\exp(0.032(V - 39.29)) + 1)$, $\alpha_n(V) = 0.038(58.58 - V)/(\exp((58.58 - V)/8.17) - 1)$, $\beta_n(V) = 0.10 \exp(-V/68)$. However the α_h was determined ad hoc, because its data were not available. The constant values in this paper were set as follows (Naitoh 1990; Hirano et al. 2005). $E_r = -30$, $E_{Ca} = +116$, $E_K = -41$,

capacity and concentration are given in ms, mV, μA/cm^2, mS/cm^2, μF/cm^2, and μM, respectively.

Mechanical Stimulation at the Dead-End and Depolarization A mechanical stimulation at the capillary dead end induces a depolarizing receptor potential from the mechano-sensitive channels distributed on the anterior part of the somatic membrane. This potential change in the local membrane area spreads to the entire membrane almost instantaneously (Dunlap 1977) as a result of the successive induction of the outward capacitive current passing through the non-depolarized adjacent membrane. In our model, the resultant current on the ciliary membrane is implemented as $I_M = I_{\mathrm{mech}}(>0)$ in (1) when the organism is at the capillary dead end ($x = 0$); otherwise, $I_M = 0$.

Relationship Between Intraciliary Ca^{2+} Concentration and Swimming Velocity The swimming velocity (v) against the intraciliary free Ca^{2+} concentration ($[Ca^{2+}]_i$) has been obtained in chemically skinned cells (Naitoh and Kaneko 1972), where $[Ca^{2+}]_i$ during normal forward swimming is 10^{-2} μM and the sign of the swimming velocity switch is near 1 μM. Figure 1.9b shows the $[Ca^{2+}]_i$ versus v graph that is used in the simulation and was determined by reference to the experimental data in (Naitoh and Kaneko 1972), but the velocity (v) was rescaled by a factor of ten because the velocity of the normal forward-swimming specimen is expected to be 1–2 mm/s (Kunita et al. 2014).

Estimation of Intraciliary Ca^{2+} Concentration In contrast to the membrane potential, calcium ions cannot traverse between cellular body and cilia because of cytoplasm buffer action (Naitoh 1990). Extraciliary Ca^{2+} passes only through the ciliary membrane. On the other hand, the Ca^{2+} are exported to the outside by calcium pumps on the ciliary membrane. So, we assumed that $[Ca^{2+}]_i$ is approximately determined by the contributions of Ca^{2+} channels and Ca^{2+} pumps on the ciliary membrane and is simply estimated by the following equation:

$$\frac{d\left[\mathrm{Ca}^{2+}\right]_i}{dt} = -\frac{I_{\mathrm{Ca}} - I^0_{\mathrm{Ca}}}{2F \times 10^3}\gamma_{\mathrm{sv}} - \frac{\left[\mathrm{Ca}^{2+}\right]_i - [\mathrm{Ca}]^0_i}{1 + \left(\left[\mathrm{Ca}^{2+}\right]_i - [\mathrm{Ca}]^0_i / K_m\right)}\gamma_{\mathrm{pu}} \tag{1.6}$$

where F is Faraday constant [C/mol], γ_{sv} is a constant proportional to the surface-to-volume ratio of the cilium, and γ_p[ms^{-1}] is a rate constant depending on the Ca^{2+} pump performance. K_{m} [μM] is the concentration at which the pump operates at half its maximum rate, and I^0_{Ca} and $\left[\mathrm{Ca}^{2+}\right]^0_i$ are the small constants of Ca^{i} Ca^{2+} current and $[Ca^{2+}]_i$ during the ordinary forward swimming, respectively.

$E_L = -25, V_{\bullet} = E_{\bullet} - E_r$. $\overline{g}_{Ca} = 0.67$, $\overline{g}_{Ca} = 1.34$, $\overline{g}_L := \left(g^{\infty}_{Ca}(0)V_{Ca} + g^{\infty}_K(0)V_K\right)/V_L$, $C_m = 1.0$. Ca K

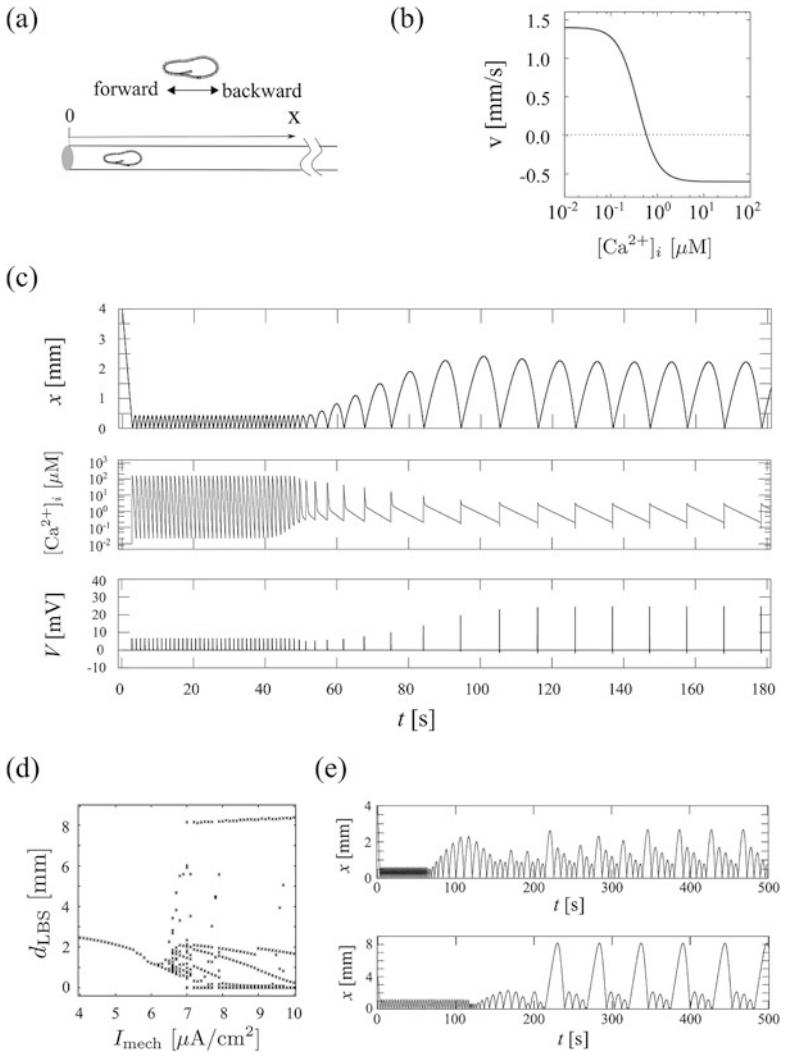

Fig. 1.9 A demonstration of the emergent behaviour (LBS) by the *Paramecium* model in the narrow dead-end capillary tube. (**a**) Experimental setting. The *Paramecium* is confined in the glass capillary tube (0.08 mm diameter, 40–50 mm length) of which the end was closed with mineral oil (Kunita et al. 2014). (**b**) Intraciliary Ca^{2+} concentration versus swimming velocity. $v = v_{-\infty} + (v_{\infty} - v_{-\infty})/(1 + b[\mathrm{Ca}]^{-a})$ (mm/s) where $v_{-\infty} = 1.40$, $v_{\infty} = \mathrm{i}\ -0.60$, $a = 2.0$, $b = 2.91 \times 10^{-7}$. (**c**) Time courses of the position of *Paramecium* (*upper panel*), membrane potential (*middle panel*), and intraciliary Ca^{2+} consecration. The *vertical lines* through the three panels indicate the timings when the organism reached the dead end ($x = 0$) with forward swimming. $I_{\mathrm{mech}} = 5$, $K_{\mathrm{m}} = 1 \times 10^2$, $\gamma_{pu} = 1.0 \times 10^{-2}$, and $\gamma_{sv} = 10 \times$ area/vol, where area/vol $= 2.0 \times 10^8$ (cm^2/l) is the surface:volume ratio of a cilium (Machemer 1974). (**d**) A bifurcation diagram of the backward distance (d_{LBS}) during the LBS period depending on the sensitivity to mechanical stimulation (I_{mech}). (**e**) Time courses of the position of the *Paramecium* model with $I_{\mathrm{mech}} = 6.6$ (*upper panel*) and 7.7 (*lower panel*). In all simulations, the time constant of the slow-inactivated Ca^{2+} channel was set to be 2000 times that of the ordinary channel

Slow Ca^{2+} Current Induced by Repetitive Stimulation The small-amplitude, long-lasting inward Ca^{2+} currents mentioned by Naitoh (1990) are assumed to be recruited by repetitive collisions at the forward end of the capillary tube. This is implemented in our model in that an ordinary Ca^{2+} channel on the ciliary membrane is modified to a slow inactivated one with a probability δp every time an action potential happens, although it becomes normal again exponentially over time.[2]

Simulation Results Figure 1.9c shows an example of the time course of the position and the ciliary electrophysiological states of the *Paramecium* model. The organism showed LBS after SBS ($t < 50$s) via a gradual increase of the backward swimming distance (upper panel of Fig. 1.9c). The shift from SBS to LBS was caused by the interaction between the organism and environment via the collision-induced recruitment of the slow-inactivated ciliary Ca^{2+} channel. Because the temporal position and the electrochemical states of the organism are determined by both the internal dynamics (Eqs. 1.1–1.6) and the history of the collision with the tube end, the model behaviour can be more diverse than that derived from only the internal dynamics. Our model includes several parameters of which values may vary through the specimen or environmental uncertain factors. I_{mech} is one of such parameters and it relates to the sensitivity to mechanical stimulations. Figure 1.9d shows dependence of backward distances after emergence of LBS on I_{mech}. As an increase of I_{mech}, various qualitative changes occurred, including doubling of the backward distances, sudden occurrence of a new long backward distance, intervening of SBS-like behaviour, and so on. Such a bifurcation diagram suggests the behaviour during LBS can vary from simple to complex and vice versa dependent on specimens and environmental details. Actually, several variants of the LBS behaviour were observed in our experiments. However, the quantitative analysis of such aspects are still not developed. Further investigation is left for future studies.

1.4 Comparative Remarks in Single-Celled Organisms and Higher Organisms

Although the capacity of memory and learning is generally assumed to be limited to higher organisms, a number of studies have suggested that these attributes can also be displayed by unicellular organisms (Bray 2009; Eisenstein 1975; Corning and Von Burg 1973; Reid et al. 2012, 2013; Hinkle and Wood 1994) as well as by nonliving systems such as spin echo and electronic circuits (Pershin et al. 2009;

[2] In the simulation, the δp assumed to be proportional to $n_s(n - n_s)/n^2$ so that n_s grows and saturates in a sigmoidal manner, where n is the total number of the Ca^{2+} channels and n_s is the number of the modified channels. The update equation for $p = n_s/n$ is given as $p(t) = (p(t_i) + k_p p(t_i)(1 - p(t_i)))$ $\exp(-(t - t_i)/\tau_p) + p_\varepsilon$, where t_i is the most recent collision time, $0 < k_p \leq 1$ is a growth rate, τ_p is a relaxation time constant, and p_ε is a sufficiently small positive constant.

Chung and Choe 2009). This perception is supported by the fact that unicellular organisms have survived successfully for billions of years. It is thus reasonable to expect a primitive form of memory and learning in the most elementary living systems. The study of the extent to which this capacity is possessed by unicellular organisms, and the manner by which it is realized, is interesting with respect to the evolutionary origin of intelligence.

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References

Applewhite P (1979) Learning in protozoa. In: Biochemistry and physiology of protozoa, vol 1. Academic Press, New York, pp 341–353

Ball P (2008) Cellular memory hints at the origins of intelligence. Nature (Lond) 451:358

Bramstedt F (1935) Dressurversuche mit paramecium caudatum und stylonychia mytilus. Z Vergl Physiol 22:490–516

Bray D (2009) Wetware: a computer in every living cell. Yale University Press, New Haven

Chung JR, Choe Y (2009) Emergence of memory-like behavior in reactive agents using external markers. In: 21st IEEE international conference on tools with artificial intelligence, pp 404–408

Church RM (1978) The internal clock. In: Hulse SH, Fowler H, Honing WK (eds) Cognitive processes in animal behavior. Erlbaum, Hillsdale

Corning WC, Von Burg R (1973) Protozoa. In: Corning WC, Dyal JA, Willows AOD (eds) Invertebrate learning. Plenum Press, New York, pp 49–122

Dunlap K (1977) Localization of calcium channels in paramecium caudatum. J Physiol 271:119–133

Eckert R, Brehm P (1979) Ionic mechanisms of excitation in *Paramecium*. Annu Rev Biophys Bioeng 8:353–383

Eisenstein EM (1975) Aneural systems and neurobiology: a point of view. In: Eisenstein EM (ed) Aneural organisms in neurobiology. Plenum Press, New York, pp 1–4

Gould JL, Gold CG (1988) The honey bee (Scientific American Library, vol 186). Freeman, San Francisco

Hinkle DJ, Wood DC (1994) Is tube-escape learning by protozoa associative learning. Behav Neurosci 108:94–99

Hirano A, Tsuji T, Takiguchi N, Ohtake H (2005) Modeling of the membrane potential change of paramecium for mechanical stimuli. Trans SICE 41:351–357

Jennings H (1906) Behavior of the lower organisms. Indiana University Press, Bloomington

Kunita I, Sato S, Saigusa T, Nakagaki T (2013) Ethological response to periodic stimulation in *Chara* and *Brepharisma*. In: Natural computing and beyond, Proceedings in Information and Communications Technology (PICT), vol 6. Springer, New York, p 3–3

Kunita I, Kuroda S, Ohki K, Nakagaki T (2014) Attempts to retreat from a dead-ended long capillary by backward swimming in *Paramecium*. Front Microbiol 5:270. doi:10.3389/fmicb.2014.0027

Machemer H (1974) Frequency and directional responses of cilia to membrane potential changes in *Paramecium*. J Comp Physiol 92:293–316