Electrical Conduction and Behaviour in 'Simple' Invertebrates

EDITED BY

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CLARENDON PRESS · OXFORD
1982

Oxford University Press, Walton Street, Oxford OX2 6DP
London Glasgow New York Toronto
Delhi Bombay Calcutta Madras Karachi
Kuala Lumpur Singapore Hong Kong Tokyo
Nairobi Dar es Salaam Cape Town
Melbourne Wellington
and associate companies in
Beirut Berlin Ibadan Mexico City

Published in the United States by Oxford University Press, New York

British Library Cataloguing in Publication Data

Electrical conduction and behaviour in 'simple' invertebrates.
1. Protozoa 2. Nervous system
I. Shelton, G.A.B.
593.1'041'88 QL921

ISBN 0-19-857171-2

Phototypesetting by Parkway Group, London and Abingdon
Printed in Great Britain
at the University Press, Oxford
by Eric Buckley
Printer to the University.

Preface

This book describes our current understanding of behavioural control mechanisms in 'simple' invertebrates other than arthropods, annelids, and molluscs. Some explanation of this unusual choice of subject matter may be needed.

Our understanding of the workings of the nervous system is substantial but incomplete. Neurophysiologists have directed much effort towards study of 'the vertebrate nervous system', usually that of frogs, dogs, cats, rats, and monkeys and this is understandable since humans are vertebrates. The complexity of 'the vertebrate nervous system', however, has made many of the results of these studies difficult to interpret. Technical and legal limitations, moreover, can present substantial obstacles to progress. Thus, while we have learned a great deal from studies of vertebrate neurophysiology and behaviour it is not surprising to find a growth of interest in parallel studies of the invertebrates. There seems little doubt that vertebrates arose from invertebrate ancestors and that some invertebrate species offer very promising experimental material. The presence of 'primitive' features is often difficult to demonstrate but many invertebrate species at least appear to be more simply organized with nervous systems containing fewer cells and behaviour which is more stereotyped. Some (the squid is a good example) possess unusually large and accessible neurons, making it possible to use experimental procedures that could not be performed on the tiny myelinated neurons of the vertebrate brain.

If one is to study invertebrate behaviour and neurophysiology, two important questions arise. Which invertebrates have the closest vertebrate affinities, the study of which may aid directly our understanding of vertebrate behavioural control? Which invertebrates have anatomical and behavioural features that make them particularly amenable to study by neurophysiologists? These two categories do not always overlap, unfortunately, and to appreciate this it is necessary to consider the organization of the animal kingdom. The classification of animals is based on evidence derived from many sources, e.g. comparative anatomy, comparative physiology, fossil history, development, and biochemistry. Not unexpectedly, the divisions which emerge do not have sharp boundaries and interpretations of the relationships between animal groups are subject to revision. It is possible, however, to make some useful generalizations. Few would dispute the basal position of the Protozoa notwithstanding the fact that some modern ciliates, for example, have become very specialized within the limitations of the protozoan body-plan. The Mollusca, the

Annelida, and the Arthropoda form a coherent group with many anatomical, physiological and developmental features in common. Their central nervous systems, for instance, are ventral, based on a segmental plan, and contain large, 'identifiable' neurons which are connected together to form pathways which are functionally the same in every individual of a species. The insects represent the pinnacle of this group. A second group of obviously related animals comprises the Chordata, the Hemichordata, the Cephalochordata, the Urochordata, and the Chaetognatha together with the Echinodermata. The pinnacle of this group is the mammalian vertebrates. These two groups apparently diverged at an early stage in evolutionary history and are recognizable from fossil records more than 500 million years old. The other groups of animals are often more difficult to fit into a simple classification and there is dispute about many of their evolutionary relationships.

Conservative estimates put the number of living animal species at well over one million but only a hundred or two have been examined in any detail by behavioural neurobiologists. Of these, a mere handful has attracted the lion's share of the experimental interest. There are many well-known reasons for this neglect of 99.99 per cent of the animal kingdom. Among the more convincing ones are ready availability from culture of certain species, economic considerations, good background literature, and ease of experimentation. It would seem reasonable, however, to look more widely in the animal kingdom for experimental material and the object of this volume is to encourage comparative physiologists to do so. Among the invertebrates this really means looking at animals which are not molluscs, annelids, or arthropods since these are already receiving intense and profitable study. By contrast to these, species directly on the chordate line of evolution together with others of less certain phylogenetic position but with promising experimental possibilities have been under-exploited. The chapters in this book aim to redress the balance. They have been chosen because the animals they discuss show a 'simplicity' (either real or superficial) of behaviour and neural organization. It may, therefore, be possible to explain their behaviour in terms of electrical activity in nerves and other excitable tissues in detail which is not yet possible in more 'complex' examples. Coverage of the animal kingdom is still not, of course, uniform or comprehensive and many small phyla have been omitted where no recent information is available.* Each of the chapters has been written by a currently-active research worker and has been designed to include reference to the latest experimental results. In many cases this includes previously unpublished material. Recent literature has been reviewed together with much older work where this is relevant and not widely known. Each contributor has attempted to show the

* Several chapters are devoted to Coelenterata including an entire chapter on a single species. This has arisen partly as a result of the unashamed bias of the editor and partly as a reflection of the current research interests of others in the field.

strengths and weaknesses of his material, to point out unusual or poorly-known behavioural characteristics, and particularly to show where further experimentation could be fruitful.

A great diversity of experimental approaches is described. These range from the more traditional comparative anatomy and behaviour to the use of biochemical and extracellular and intracellular electrical recording techniques to examine behaviour at the cell level. These approaches have been used to investigate such topics as the capacity and versatility of 'simple' invertebrate behaviour machines; the electrical conduction mechanisms, both nervous and non-nervous, underlying behaviour in its widest sense (including such topics as control of secretion and ciliary motion); and the ultrastructure and membrane properties of sense cells, conducting elements and effectors. The results are interpreted from a zoological viewpoint.

It is a pleasure to thank Professor J. W. S. Pringle for his kindness and help. Dr Elaine Robson, Mr Dick Manuel, Mr M. C. Holley, and Dr T. D. Hughes made many useful criticisms and suggestions for improvement. The staff of the Oxford University Press have been patient and helpful at all stages of production. Miss A. M. Hillman and Mr L. G. Symonds gave valuable help with the index. To all of them and especially to the contributors I am very grateful.

GABS

Oxford
January 1982

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1. Protozoa

Yutaka Naitoh

Introduction

Protozoans are single-celled animals, ranging from 20 to 500 μm in length. They show immense variety of shape, motile activity, and way of life. The locomotion of protozoans is dependent on the activity of organelles such as cilia, flagella, pseudopods, myonemes, and/or other intracellular contractile fibres. The activities of these organelles change in response to stimuli. The protozoan accordingly exhibits behavioural responses such as acceleration or inhibition of locomotion, change in direction, change in body shape, etc., depending on the kind of organelles activated. The behavioural responses of a protozoan are so adaptive that many scientists never doubted the presence of a nerve-like system which controls its behaviour as does the nervous system of a higher multicellular animal. As a matter of fact, in some ciliates (*Euplotes* and *Stylonychia*), subcortical fibres connecting the bases of their cilia (cirri) are visible through a light microscope. In 1920 Taylor reported that incision of these fibres disrupted the synchronous reversal of beating direction in each cirrus, thus causing loss of avoiding reaction (quick backward locomotion) of the animal upon stimulation. He argued that the fibre system conveys signals to each cirrus for their co-ordinated (reversed) beating. Thus the fibre system had been regarded for almost a half century as an example of differentiation of the nervous function at the intracellular level (see Bullock and Horridge 1965).

In 1966, Okajima and Kinosita re-examined Taylor's experiments. They found no disruption of the ciliary co-ordination even after the fibres were dissected. They proposed, therefore, that the spread of an electric impulse such as a receptor potential through the cell membrane is a possible way of signalling for co-ordinated reversed beating in spatially separated cirri. Their hypothesis was electrophysiologically supported by Naitoh and Eckert (1969*b*), who demonstrated that the inside of the protozoan cell was electrically isopotential. This means that electric impulses elicited at any region of the protozoan membrane spread instantaneously all over the cell membrane. They further demonstrated that incision of the cell did not destroy its isopotential nature. An idea similar to that of Okajima and Kinosita had been proposed by Worley as early as 1934 based on his microdissection experiments on *Paramecium*. He found that reversal of ciliary beating took place simultaneously in all the cilia on the incised animal, provided a small portion of the membrane remained intact so as to make a bridge between both sides of the incision. This important finding had

long been overshadowed by Taylor's fascinating, but false, conclusion which ignored the traumatic effect of incision on cellular motility; the sensitivity of the cirri to produce reversed beating is much retarded by incision.

Some physiologists of the early days (cf. Verworn 1896) had understood that the reversed beating of cilia upon stimulation is a kind of cellular excitation, because reversed beating occurs on the cell surface facing the cathode when a ciliate is placed in an electric field. This fact apparently obeys 'Pflüger's law of polar excitation' held for excitable tissues such as nerves and muscles. Much work on ciliary reversal, therefore, has been carried out to solve the mechanism for cellular excitation.

In his pioneering measurements of the membrane potential of *Paramecium* by an intracellular microcapillary electrode Kamada (1934) demonstrated non-selective cationic permeability in the resting membrane. Perturbation of the membrane potential and its behavioural correlate were demonstrated first by Kinosita (1954) in a parasitic ciliate *Opalina*. His simultaneous cinematographic recordings of the membrane potential and the movement of cilia revealed that a calcium-dependent membrane depolarization was always accompanied by ciliary reversal. More recently, Naitoh and Eckert (1974; see also Eckert and Naitoh 1972; Eckert 1972; Naitoh 1974; Eckert, Naitoh, and Machemer 1976) demonstrated that the ionic mechanisms for ciliate membrane electrogenesis are essentially identical with those for other excitable cells.

Certain regions of protozoan membrane (receptor regions) respond to certain stimuli by generating a receptor potential. The receptor potential spreads electrotonically all over the cell due to its isopotential nature, and thereby activates (opens) voltage-sensitive ionic channels of the membrane. Ions consequently move down an electrochemical gradient through the activated channels, producing an action potential together with a transient change in the intracellular concentration of the ions, which in turn modifies the motile activity of the animal. For example, a depolarizing action potential of ciliate protozoans is mediated by the inflow of calcium ions through voltage-sensitive calcium channels in the ciliary membrane. Thus the action potential produces an increase in calcium concentration in the cilia, which activates the calcium-sensitive reversal mechanism in the cilia, causing backward movement of the animal (Naitoh and Kaneko 1972, 1973). The calcium-mediated mechanism for the coupling between membrane electrogenesis and the motile activity of the cell is analogous to that for the 'excitation-contraction coupling' in skeletal muscle. Membrane hyperpolarization causes an increase in the frequency of ciliary beat in the normal direction, through a mechanism not clarified yet, bringing about rapid forward movement of the specimen (escape reaction).

The anterior end of *Paramecium* (and some other ciliates) produces a depolarizing receptor potential while the posterior end produces a

hyperpolarizing receptor potential when stimulated mechanically. If both ends are stimulated simultaneously, the decision whether the specimen goes forward or backward is dependent exclusively on the membrane potential determined by summation of the two opposite receptor potentials. When it is depolarized, the animal goes backward in association with generation of a calcium-dependent action potential, while it goes forward with increased swimming velocity when hyperpolarized. This phenomenon is analogous to the neural integration found in a single neuron of higher animals. Protozoans, therefore, deserve the nickname 'swimming neuron' as well as 'swimming receptor'. Protozoans can easily be manipulated genetically to obtain mutants useful for specific experimental purposes. Some mutants of *Paramecium* (and of *Tetrahymena*) which show abnormal swimming behaviour have been found to possess electrogenetic malfunctions in their membrane (Kung and Eckert 1972; Takahashi and Naitoh 1978). *Paramecium* and *Tetrahymena* are easily grown as a mass clonal culture, offering enough membrane material for its conventional biochemical analysis. Comparison of the membrane between normal and mutant specimens is providing useful information for identifying the protein corresponding to the ionic channel. The combination of genetics, electrophysiology and biochemistry of protozoans shows great promise for better understanding of the molecular mechanisms for membrane excitation and its behavioural correlates in the animal kingdom. The following sections describe in more detail the recent progress which has been made in these fields.

1.1 Ciliary motion and swimming behaviour in ciliates

Locomotor behaviour of ciliates depends mostly on their ciliary motion. The direction of the effective stroke of the body cilia in non-stimulated *Paramecium* is slightly right posterior (Párducz 1967; Machemer 1972) (Figs. 1.1 and 1.2(c)). The ciliary stroke, therefore, drives the specimen forward with left-handed rotation around its longitudinal axis, while beating of its oral cilia drives the anterior part of the specimen away from the oral side. The swimming path of the specimen, consequently, becomes left spiral (Fig. 1.2(a)).

When the direction of the effective stroke shifts clockwise to be more parallel with the anteroposterior axis of the specimen, it swims forward and faster with increased pitch of its spiral path (Fig. 1.2(d)).

A counterclockwise shift, however, results in a decrease in the forward-swimming velocity together with a decrease in the pitch. When the shift is so great that the direction of the effective stroke is about 90° to the anteroposterior axis, the ciliary beat gives the specimen its rotation around the longitudinal axis only and not its forward momentum. The specimen thus gyrates about its posterior end (Fig. 1.2(b)). The specimen swims backward

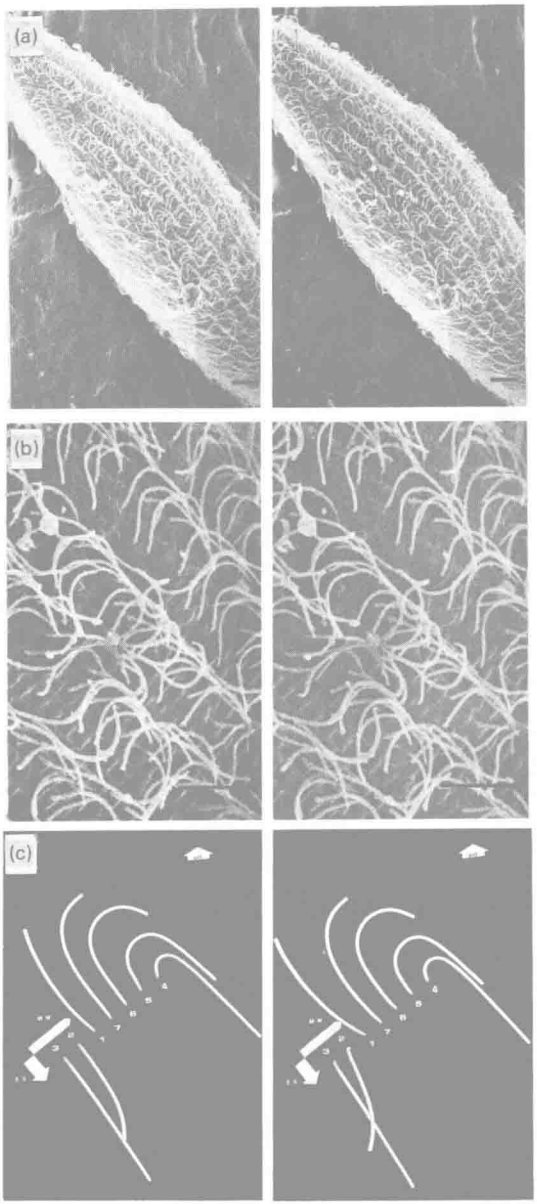

Fig. 1.1. (a) and (b): Surface view of an instantaneously fixed forward-swimming specimen of *Paramecium caudatum*. Black lines on the photographs correspond to 10 μm, (Photographs by courtesy of A. S. Baba.) (c): Stereophotograph of wire models of cilia of *Paramecium*, showing their three dimensional beating form. (The photograph was made by K. Sugino based on the data of Machemer (1972).)

with a right spiral path when the shift is over 90° (Fig. 1.2 (a)). This counter-clockwise shift in the direction of the effective stroke of the cilia in order to induce backward swimming of the specimen is termed 'ciliary reversal'.

The beat frequency of the cilia in non-stimulated *Paramecium* is 15–20 Hz. It varies from 0–50 Hz in accordance with the physiological condition of the cell (Machemer 1974; Machemer and Eckert 1975; Brehm and Eckert 1978). An increase in beat frequency of cilia together with a slight clockwise shift in the direction of the effective stroke, which causes an increase in forward swimming velocity of the specimen, is termed 'ciliary augmentation'.

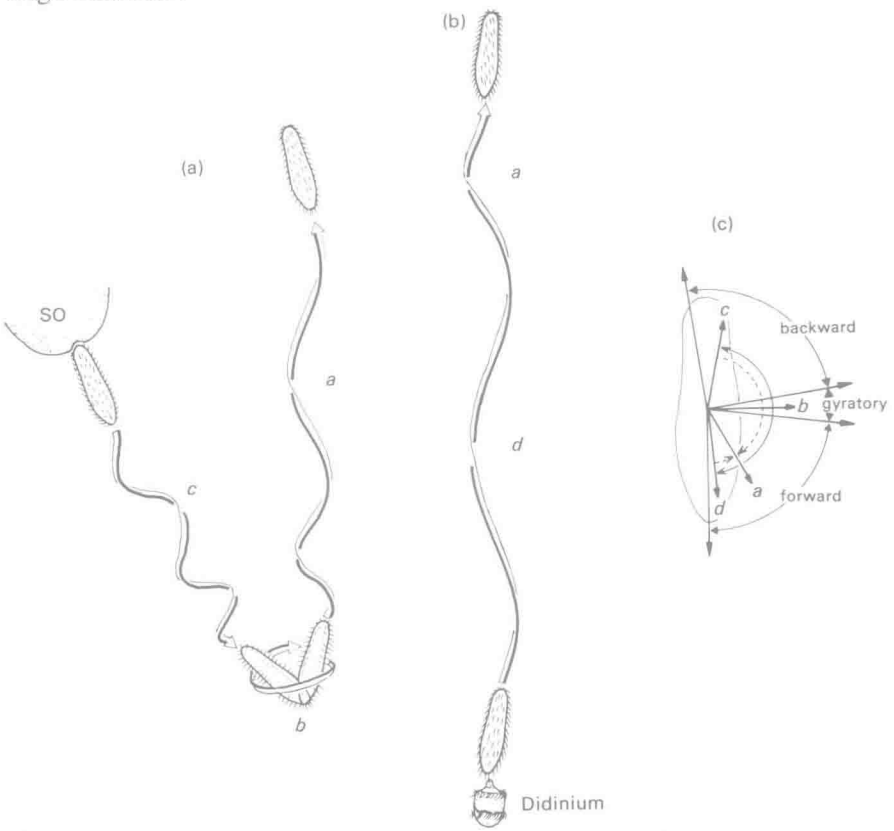

Fig. 1.2. Schematic representations of behavioural responses in *Paramecium*. (a): Avoiding reaction upon bumping against a solid object (SO) with its anterior end. It shows backward swimming first *c*, then gyrates about its posterior region *b*, and finally resumes normal forward locomotion *a*. (b): Escaping reaction shown when it is attacked by its predator (e.g. *Didinium*) from behind. It increases forward swimming velocity for a moment *d*, then resumes normal forward swimming *a*. (c): Approximate direction of effective stroke of cilia corresponding to each phase of the behavioural reactions (surface view).

1.2 Behavioural responses in ciliates

1.2.1 Mechanical stimulation

When a forward swimming *Paramecium* bumps its anterior end against an obstacle, ciliary reversal takes place, so that the specimen swims backward (Fig. 1.2(a)). The reversed cilia soon begin to shift their direction of effective stroke clockwise to resume their original right-posterior direction. The backward swimming speed decreases, therefore, and the specimen halts its swimming for a while and then regains its forward momentum. Since the anterior part of the specimen gyrates about its posterior end at the moment of backward–forward switch (Fig. 1.2(b)), the new forward swimming direction is usually different from that before collision (Fig. 1.2(a)). Thus the specimen avoids the obstacle and continues its forward swimming. This behavioural response is called 'avoiding reaction' (Jennings 1906). If the specimen encounters the obstacle again, it repeats the reaction until it can avoid the obstacle.

An avoiding reaction is usually completed in a fraction of a second. A stronger collision (collision with a solid object at a right angle) causes larger changes in both direction of effective stroke and the beat frequency of the cilia, which result in the specimen swimming backward for a longer distance. A minor collision (collision with a soft object at a small angle) causes only a brief halting of the specimen.

When a predator of *Paramecium* (carnivorous rotifers, *Didinium*, newly hatched small fish, etc.) touches the posterior portion of *Paramecium*, ciliary augmentation takes place so that the specimen swims forward faster than its normal swimming speed to escape from the attack by the predator (Fig. 1.2(b)). This behavioural response is called the 'escaping reaction' (Naitoh 1974). It is more marked and lasts longer when the mechanical touch by the predator is stronger.

The escaping reaction of *Paramecium* is seen in culture when the specimen is trapped in a narrow chink of debris. A scratch on the thicker posterior portion of the specimen by the wall of the chink causes ciliary augmentation and struggling against the chink until the specimen swims out of it. A light tap to the culture vessel also induces the escaping reaction in all the specimens in the culture. The avoiding reaction and the escaping reaction can also be induced by touching the anterior or posterior region of a specimen with the tip of a small glass needle (Doroszewski 1961; Naitoh and Eckert 1969*a*).

1.2.2 Chemical stimulation

When forward swimming specimens of *Paramecium* in 1 mM $CaCl_2$ solution encounter a small drop of 10 mM $CaCl_2$ solution they all show avoiding reactions and leave the drop empty of specimens (negative chemokinesis; Fraenkel and Gunn 1961), but when specimens in 10 mM $CaCl_2$ solution

come in contact with a drop of 1 mM $CaCl_2$ solution they show escape reactions and enter the drop quickly. They swim forward through the drop to reach the border with the surrounding 10 mM $CaCl_2$ solution where they show the avoiding reaction. Once the specimens have entered the drop, therefore, they cannot leave (positive chemokinesis).

The reaction exhibited by the specimens at the border between two solutions becomes less conspicuous with time after the drop is introduced. The concentration gradient of $CaCl_2$ at the border is assumed to become less steep with time by diffusion.

On the other hand, sudden transfer of the specimen from 1 mM $CaCl_2$ solution to 10 mM $CaCl_2$ solution does not produce an avoiding reaction in the specimen. These facts suggest that an essential factor for initiation of avoiding reactions at the border is the concentration gradient along the longitudinal axis of the specimen and not the time change in the concentration at a certain part of the specimen.

It is noteworthy that initiation of the behavioural responses at the border is independent of cation species. An avoiding reaction occurs when the specimen meets with a concentrated area, while an escape reaction takes place when it encounters a dilute area, though the effectiveness for inducing the response is different in different cation species.

The reaction to hydrogen ions is somewhat peculiar. When the specimens in a solution of pH 6 encounter an area of different pH they always show an avoiding reaction. Consequently, when a drop of diluted acid is placed in a thin suspension of *Paramecium* on a glass plate all the specimens are trapped in an area, strength pH 6, around the acid drop (Jennings 1906; Dryl 1974).

Behavioural responses exhibited by the specimens at a chemical border can be mimicked by applying chemicals to a localized area on the cell surface through a micropipette (Fig. 1.3; Naitoh 1961). *Paramecium* and many other ciliates show long-lasting backward swimming (for scores of seconds) upon transfer from a medium with a low $[K^+]/\sqrt{[Ca^{2+}]}$ ratio to a medium with a high $[K^+]/\sqrt{[Ca^{2+}]}$ ratio. By contrast to the avoiding reaction at a chemical border, initiation of the long-lasting ciliary reversal is dependent on the ratio and not the ionic concentration (Jahn 1962; Naitoh and Yasumasu 1967; Naitoh 1968).

1.2.3 Electric stimulation

When an electric current is applied to a medium where specimens of *Paramecium* are swimming in random directions, they suddenly turn to swim toward the cathode. The behavioural response is termed 'galvanotaxis'.

The galvanotactic response depends on the response of the cilia to electric current. Cilia on the cell surface closer to the anode, where electric current enters the cell, show ciliary augmentation, while cilia on the surface closer to

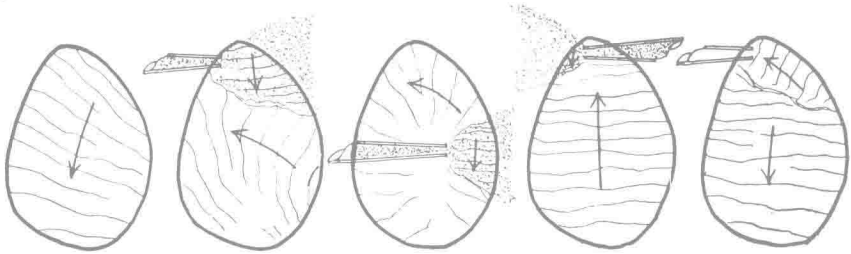

Fig. 1.3. Ciliary responses of *Opalina* in Ringer solution to localized application of 15 mM KCl solution to various regions ((b): anterior right; (c): posterior left; (d): anterior left) of its ventral surface. (a): Control. (e): Ciliary response upon cessation of the localized application of the KCl solution to the anterior right region b. Arrows indicate the direction of propagation of metachronal wave, which approximates to the direction of the effective stroke of the cilia. The pictures are viewed from the dorsal side. Metachronal wave lines can be seen through the transparent cytoplasm of the specimen (Naitoh 1961).

the cathode, where electric current leaves the cell, show ciliary reversal (Ludloff 1895; Jahn 1961). These two opposite ciliary responses in a single specimen yield a torque which rotates the specimen to point toward the cathode. Since the area which shows ciliary reversal is smaller than that which shows ciliary augmentation, the cathodally-oriented specimen swims towards the cathode.

Galvanotactic orientation of ciliates is not always toward the cathode, though ciliary reversal occurs on the cathodal side with few exceptions. They sometimes orient themselves obliquely to the electric field (Dryl 1963) or even toward the anode (Wallengren 1903), depending on their cellular shape, distribution of cilia or cirri and on the topographical differences in the sensitivity of cilia to electric current.

Topographical differences in the electric threshold for initiation of ciliary reversal on the cell surface of *Opalina* can be detected by localized application of electric current through a microelectrode, the tip of which is placed close to the cell surface (Okajima 1953). The right anterior portion of *Opalina* is the most sensitive to electric current (Fig. 1.4).

1.2.4 Other stimuli

When a suspension of *Paramecium* is introduced into a long glass tube, the specimens swarm in the upper portion of the tube (negative geotaxis: Verworn 1896). The negative geotaxis becomes conspicuous if the specimens are stimulated so as to increase their forward swimming velocity. On the other hand, well-fed specimens containing many food vacuoles in their posterior region exhibit a more marked negative geotaxis than starved specimens without food vacuoles (Fig. 1.5). These facts suggest that upward orientation of the specimens is due to their heavier posterior. This, com-

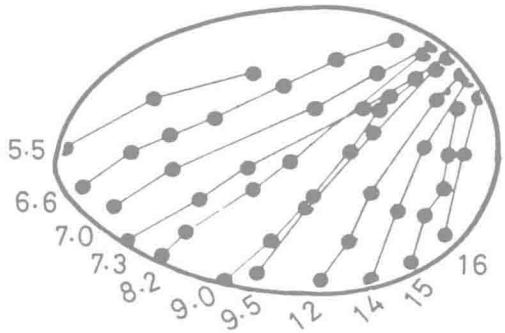

Fig. 1.4. The 'equithreshold lines' on the ventral surface of *Opalina* (viewed from its dorsal side). The numerals indicate arbitrary values of the cathodic current to evoke just perceptible ciliary reversal on the areas (●) where the tip of an extracellular microelectrode is placed. The anterior right region has the highest sensitivity to cathodic current (Okajima 1953).

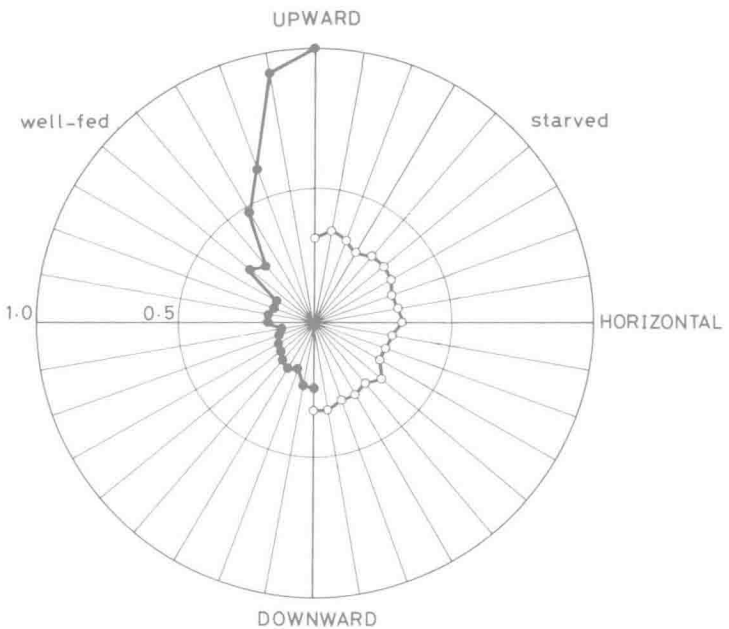

Fig. 1.5. Circular graphic representation of geotactic orientation in *Paramecium caudatum*. Specimens which swim in a certain angle to the horizontal plane were counted 1 min after they were introduced into a thin vertical vessel (4 cm in width, 30 cm in height, 2 mm in depth), and plotted on a radius corresponding to the angle. Upward orientation predominates in well-fed specimens (●), while starved specimens orient themselves in random direction (○). Number of specimens showing upward orientation is regarded as unity (Fukui 1980).