The physiology of excitable cells

FOURTH EDITION

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Introduction

Suppose a man has a tomato thrown at his head, and that he is able to take suitable evasive action. His reactions would involve changes in the activity of a very large number of cells in his body. First of all, the presence of a red object would be registered by the visual sensory cells in the eye, and these in turn would excite nerve cells leading into the brain via the optic nerve. A great deal of activity would then ensue in different varieties of nerve cell in the brain and, after a very short space of time, nerve impulses would pass from the brain to some of the muscles of the face and, indirectly, to muscles of the neck, legs and arms. The muscle cells there would themselves be excited by the nerve impulses reaching them, and would contract so as to move the body and so prevent the tomato having its intended effect. These movements would themselves produce excitation of numerous sensory endings in the muscles and joints of the body and in the organs of balance in the inner ear. The resulting impulses in sensory nerves would then cause further activity in the brain and spinal cord, possibly leading to further muscular activity.

A chain of events of this type involves the activity of a group of cell types which we can describe as 'excitable cells': a rather loose category which includes nerve cells, muscle cells, sensory cells and some others. An excitable cell, then, is a cell which readily and rapidly responds to suitable stimuli, and in which the response includes a fairly rapid electrical change at the cell membrane.

The study of excitable cells is fascinating for a number of reasons. These are the cells which are principally involved in the behavioural activities of animals, including ourselves: these are the cells with which we move and think. Yet just because their functioning must be examined at the cellular and subcellular levels of organization, the complexities that emerge from investigating them are not too great for adequate comprehension. So it is frequently possible to pose specific questions as to their properties and to elicit some of the answers to these questions by suitable experiments. It is perhaps for this reason that the subject has attracted some of our foremost scientists. As a consequence, the experimental evidence on which our knowledge of the physiology of excitable cells is based is often elegant, clear-cut and intellectually exciting, and frequently provides an object lesson in the way a scientific investigation should be carried out. Nevertheless, there are very many investigations still to be done in this field, many questions which have yet to be answered, and undoubtedly very many which have not yet been asked.

Most readers of this book will possess a considerable amount of information on basic ideas in the biological and physical sciences. But it may be as well in this introductory chapter to remind them, in a rather dogmatic fashion, of some of the background which is necessary for a more detailed study: to formulate, in fact, a few axioms.

The biological material
Cells

All large organisms are divided into a number of units called cells, and every cell is the progeny of another cell. This statement constitutes the cell theory. Every cell is bounded by a cell membrane and contains a nucleus in which the genetic material is found. The main part of the living matter of the cell is a highly organized system called the cytoplasm, which is concerned with the day-to-day activity of the cell. The cell membrane separates this highly organized system inside from the relative chaos that exists outside the cell.

In order to maintain and increase its high degree of organization and in order to respond to and alter its environment, the cell requires a continual supply of energy. This energy must be derived ultimately from the environment, usually in the form of chemical energy such as can be extracted by the cell from glucose molecules. We can describe the cell in thermodynamic terms as an open system maintained in a rather improbable steady state by the continual expenditure of energy. Its life is a continual battle against the second law of thermodynamics (which we may state without gross inaccuracy as 'things tend to get mixed up').

The cells of nervous systems are called *neurons*. Their primary function is the handling of information. Within the cell this mainly takes the form of changes in the electric

potential across the cell membrane, whereas information is passed between cells largely as chemical messages.

The idea that the nervous system is composed of discrete cells is known as the neuron theory. This view, which is simply a particular application of the cell theory, was developed during the nineteenth century and is now generally accepted (see Shepherd, 1991). The alternative proposal, that nervous systems are not divided into separate membrane-bounded entities (the reticular theory), was difficult to reconcile with the observations of light microscopists, and seems to be conclusively refuted by the evidence of electron microscopy.

Neurons have functional regions specialized for different purposes. Sites where one neuron contacts another cell, usually another neuron, and transmits or receives some information, are known as *synapses*. Synaptic transmission is usually a one-way process, from the presynaptic cell to the postsynaptic cell. Areas of the neuron that receive synaptic contacts from presynaptic neurons form the *input region* (fig. 1.1). The input region commonly consists of branched processes called dendrites, and may include the surface of

the cell body (the soma, containing the cell nucleus) of the neuron. The postsynaptic responses in the input region may be sufficient to produce excitation in the *conductile region* of the neuron, whose activity consists of unitary events called nerve impulses or action potentials. The conductile region is a long process called the *axon*. The axon usually terminates in fine branches that make synaptic contact with other cells, such as other neurons or muscle cells. These terminals are presynaptic and form the *output region* of the neuron. They usually secrete a chemical substance, the neurotransmitter, when an action potential arrives along the axon, and this carries information across the synapses to the postsynaptic cell.

Proteins

So pervasive are the functions of proteins in cells that one way of defining living material is to say that it contains active proteins. Proteins are composed of chains made up from different combinations of twenty different amino acids, and their properties depend critically upon the sequence in which these amino acids are arranged.
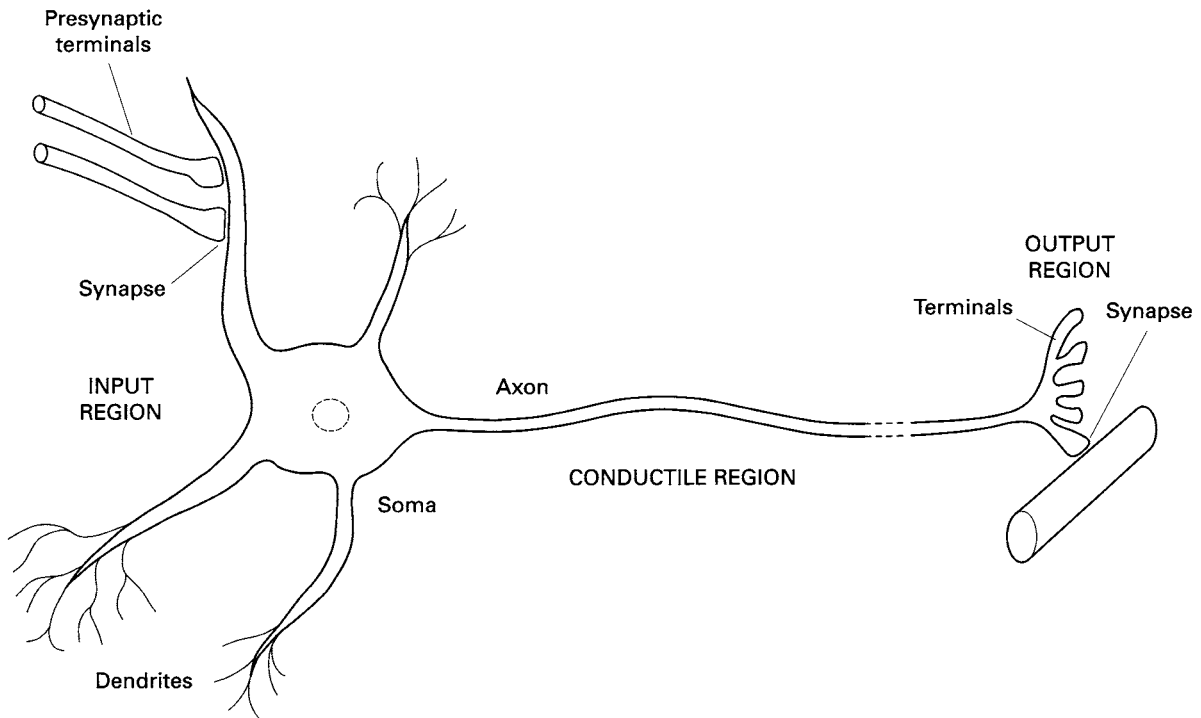
Figure 1.1. The main regions of a neuron. The input region, commonly the dendrites and soma of the cell, receives synaptic inputs from a number of different nerve fibres. The conductile region, the axon, carries all-or-nothing action potentials from the input region to the output region. The output region, the nerve terminals, forms synapses with other neurons or muscle cells. The diagram is based very loosely on a vertebrate spinal motoneuron, where the input region (soma and dendrites) is in the spinal cord, and the axon passes down the ventral root and along peripheral nerves to the output region at the neuromuscular junction.

The protein's amino acid sequence is specified by the nucleotide base sequence in the DNA molecules which form the genetic material of the cell. This means that proteins are the product of evolution, so that present-day proteins largely represent stable and successful sequences. Animals whose cells produced too many unstable or non-functional sequences would have died before producing viable offspring, so the genes specifying those sequences have mostly been eliminated. Different animal species may have proteins with only minor variants in amino acid composition. The same animal may produce a number of very similar sequences, perhaps adapted to slightly different roles in different tissues. These variants of essentially the same proteins are called *isoforms*.

The shape of many protein molecules changes when they react with smaller molecules or other proteins. Changes of this type underlie much protein activity, such as enzymic hydrolysis, opening of membrane channels, and muscular contraction. The day-to-day activity of the cell can thus be described largely in terms of the actions of proteins; the reader of modern accounts of cell biology (such as those by Albert *et al.*, 1994, and Lodish *et al.*, 1995) will find ample illustration of this statement.

Animals

Every animal has a history: every animal owes its existence to the success of its ancestors in combating the rigours of life; that is to say, in surviving the rigours of natural selection. Hence every animal is adapted to its way of life, and its organs, its tissues, its cells and its protein molecules are adapted to performing their functions efficiently.

An animal is a remarkably stable entity. It is able to survive the impact of a variety of different environments and situations, and its cells and tissues are able to survive a variety of different demands upon their capacities. An animal is a complex of self-regulating (homeostatic) systems. These systems are themselves coordinated and regulated so that the physiology and behaviour of the animal form an integrated whole.

Nervous systems

A nervous system is that part of an animal which is concerned with the rapid transfer of information through the body in the form of electrical signals. The activity of a nervous system is initiated partially by the input elements – the sense organs – and partially by endogenous activity arising in certain cells of the system. The output of the system is ultimately expressed via effector organs – muscles, glands, chromatophores, etc.

Primitive nervous systems consist of scattered but usually interconnected nerve cells, forming a nerve net, as in coelenterates. Increase in the complexity of responses is associated with the aggregation of nerve cell bodies to form ganglia and, when the ganglia themselves are collected and connected together, we speak of a *central nervous system*. The *peripheral nervous system* is then mainly composed of nerve fibres originating from the central nervous system. Peripheral nerves contain afferent (sensory) neurons taking information inwards into the central nervous system and efferent (motor) neurons taking information outwards. Neurons confined to the central nervous system are known as interneurons. Ganglia which remain or arise outside the central nervous system, and the nerve fibres which lead to and arise from them to innervate the animal's viscera, are frequently described as forming the *autonomic nervous system*.

One of the simplest, but possibly not one of the most primitive, modes of activity of a nervous system is the *reflex*, in which a relatively fixed output pattern is produced in response to a simple input. The stretch reflexes of mammalian limb muscles provide a well-known example (fig. 7.20). Stretching the muscle excites the endings of sensory nerve fibres attached to certain modified fibres (muscle spindles) of the muscle. Nerve impulses pass up the sensory fibres into the spinal cord where they meet motor nerve cells (the junctional regions are called *synapses*) and excite them. The nerve impulses so induced in the motor nerve fibres then pass out of the cord along peripheral nerves to the muscle, where their arrival causes the muscle to contract. Much more complicated interactions occur in the analysis of complex sensory inputs, the coordination of locomotion, the expression of the emotions and instinctive reactions, in learning and other 'higher functions'. These more complicated interactions are outside the scope of this book.

Electricity

Matter is composed of atoms, which consist of positively charged nuclei and negatively charged electrons. Static electricity is the accumulation of electric charge in some region, produced by the separation of electrons from their atoms. Current electricity is the flow of electric charge through a conductor. Current flows between two points connected by a conductor if there is a potential difference between them, just as heat will flow from a hot body to a cooler one placed in contact with it. The unit of potential difference is the *volt*. The current, i.e. the rate of flow of charge, is measured in *amperes*, and the quantity of charge transferred is measured in *coulombs*. Thus one coulomb is transferred by a current of one ampere flowing for one second.

In many cases it is found that the current (I) through a

Table 1.1 *Some electrical quantities and their units*

Quantity	Symbol for quantity	Unit	Symbol for unit	Equivalent
Charge	Q	coulomb	C	A s
Current	I	ampere	A	C s^{-1}
Potential difference	V, E	volt	V	J C^{-1}
Energy (work)		joule	J	C V
Power		watt	W	J s^{-1}, A V
Resistance	R	ohm	Ω	V A^{-1}
Conductance	G	siemens	S	Ω^{-1}, A V^{-1}
Capacitance	C	farad	F	C V^{-1}

Note:
It is conventional to write the symbols for quantities in italics and the symbols for units in roman type.

Table 1.2 *Some prefixes for multiples of scientific units*

Multiple	Prefix	Symbol
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{3}	kilo	k
10^{6}	mega	M
10^{9}	giga	G

conductor is proportional to the potential difference (V) between its ends. This is *Ohm's Law*. Thus if the constant of proportionality, the *resistance* (measured in *ohms*) is R, then

$$V = IR$$

The specific resistance of a substance is the resistance of a 1 cm cube of the substance. The resistance of a wire of constant specific resistance is proportional to its length and inversely proportional to its cross-sectional area. The reciprocal of resistance is called *conductance* (G).

Let us apply Ohm's law to a simple calculation. In chapter 6 we shall see that under certain conditions small channels open to let sodium ions flow through. If we can measure this current flow and we know what the driving voltage is, we can calculate the conductance of the channel. Thus in one experiment the single channel current was 1.6 pA with a driving voltage of 90 mV. (Table 1.1 shows selected electrical units and table 1.2 gives prefix names for multiples and submultiples.) Applying Ohm's law, the conductance of the channel is given by

$$G = I/V$$

i.e. conductance (siemens) $= \dfrac{\text{current (amps)}}{\text{voltage (volts)}}$

$$= \frac{1.6 \times 10^{-12}}{90 \times 10^{-3}}$$

$$= 17.8 \text{ pS}$$

The total resistance of a number of resistive elements arranged in series is the sum of their individual resistances,

whereas the total conductance of a number of elements in parallel is the sum of their conductances. A patch of membrane containing five channels each with a conductance of 17.8 pS, for example, will have a conductance of 89 pS if all the channels are open.

Two plates of conducting material separated by an insulator form a capacitor. If potential difference V is applied across the capacitor, a quantity of charge Q, proportional to the potential difference, builds up on the plates of the capacitor. Thus

$$Q = VC$$

where C, the constant of proportionality, is the *capacitance* of the capacitor. When the voltage is changing, charge flows away from one plate and into the other, so that we can speak of current, I, through a capacitor, given by

$$I = C\frac{dV}{dt}$$

where dV/dt is the rate of change of voltage with time. The capacitance of a capacitor is proportional to the area of the plates and the dielectric constant (a measure of the ease with which the molecules of a substance can be polarized) of the insulator between them, and inversely proportional to the distance between the plates. The total capacitance of capacitors in parallel is the sum of the individual capacitances, whereas the reciprocal of the total capacitance of capacitors in series is the sum of the reciprocals of their individual capacitances.

Scientific investigation
Science is concerned with the investigation and explanation of the phenomena of the natural world. Any particular investigation usually starts with an idea – a hypothesis – about the relations between some of the factors in the

system to be studied. The hypothesis must then be tested by suitable observations or experiments. This business of testing the hypothesis is what distinguishes the scientific method from other attempts at the acquisition of knowledge, and hence it follows that a scientific hypothesis must be capable of being tested. We must therefore understand what is meant by 'testing' a hypothesis.

In mathematics and deductive logic it is frequently possible to prove, given a certain set of axioms, that a certain idea about a particular situation is true or not true. For instance, it is possible to prove absolutely conclusively that, in the system of Euclidean geometry, the angles of an equilateral triangle are all equal to one another. But this absolute proof of the truth of an idea is not possible in science. For example, consider the hypothesis 'No dinosaurs are alive today'. This statement would be generally accepted by biologists as being almost certainly true. But, of course, it is just possible that there are some dinosaurs alive which have never been seen. Some years ago the statement 'No coelacanths are alive today' would also have been accepted as almost certainly correct.

However, in many cases, it *is* possible to prove that a hypothesis is false. The hypothesis 'No coelacanths are alive today' has been proved, conclusively, to be false. If we were to find just one living dinosaur, the hypothesis 'No dinosaurs are alive today' would also have been shown to be false. It follows from this argument that in order to test a hypothesis it is necessary to attempt to disprove it. When a hypothesis has successfully survived a number of attempts at disproof, it seems more likely that it provides a correct description of the situation to which it applies (Popper, 1963).

If we can test a hypothesis only by attempting to disprove it, it follows that a scientific hypothesis must be formulated in such a way that it is open to disproof – so that we can think of an experiment or observation in which one of the possible results would disprove the hypothesis. Any idea which we cannot see how to disprove is not a scientific hypothesis.

But where do the ideas come from? Science is a progressive activity. Advances are usually made step by step. Ideas arise in a controlled imagination: the scientist usually starts from a generally accepted understanding of the situation and makes a small conjecture into the unknown. A high rate of progress follows two particular types of advance: ideas which provide a major reinterpretation of what we know, and new techniques. In 1954, for example, as we shall see in chapter 19, the study of muscular contraction entered a new and highly productive phase as the result of the formulation of the sliding filament theory, which itself arose in the context of advances in X-ray diffraction methods and electron microscopy. More recently, the advent of the patch clamp technique (fig. 2.4) has led to a great flowering of work on the ionic channels of cell membranes.

What implications does the nature of science have for learning about science? Students of any subject must get to grips with its intellectual credentials, if they are to be worth their salt. For the science student, this implies that simply comprehending a proposition that we believe to be true is not enough. It is also necessary to understand why we believe it to be true, what the evidence for the proposition is, and hence what sort of evidence might lead us to revise our opinion about it.

It is for this reason that this book is much concerned with experiments and observations, and not simply with the understanding that has arisen from them. The conclusions from some of these experiments will stand the tests of further investigations in the future, those from others will have to be revised. Science students cannot hope to know everything about their subject, but if they understand just why they believe some of what they know, then they can look future in the face.