VOLTAGE-SENSITIVE ION CHANNELS

Voltage-Sensitive Ion Channels

Biophysics of Molecular Excitability

by

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To Alice, Clyde, Penny, Jeremy, Joshua and Ilana Leuchtag,
and
to the memory of my parents,
Käthe (Wagner) Leuchtag and Rudolf Wilhelm Leuchtag,
who with United States citizenship became Kathe and Rudolph Leuchtag Light

Contents

PREFACE

The goal of this book is to explore the complexity of a microscopic bit of matter that exists in a myriad of copies within our bodies, the voltage-sensitive ion channel. We seek to investigate the way in which these macromolecules make it possible for the long fibers of our nerve and muscle cells to conduct impulses. These integral components of cell membranes are marvels of nature's evolutionary adaptation. To understand them we must probe the boundaries of physics and chemistry.

Since function is intimately related to structure, we examine the molecular structure of channels, focusing on physical principles that govern all matter. With the application of genetic methods, our knowledge of ion channels has broadened and deepened. In the hope that research can help ameliorate suffering, we discuss the diseases that arise from channel malfunctions due to genetic mutations.

This book is intended for students and scientists who are willing to travel into uncharted waters of an interdisciplinary science. We approach the subject of voltage-sensitive ion channels from various points of view. This book seeks to give voice to the viewpoints of the physical and the biological scientist, and to bridge gaps in terminology and background. Readers may find this book to have both elementary and advanced aspects: For the reader trained in the biological sciences, it reviews background in physics and chemistry; for the reader trained in the physical sciences, it reviews background in physiology and biochemistry. Beyond the introductory chapters, we follow up concepts that may be as new and challenging to you, the reader, as at first they were to me.

Ten years or so ago at a Biophysical Society meeting I was talking to a fellow channel scientist, one considerably younger than I. I happened to mention that, in my opinion, voltage-sensitive ion channels will eventually have to be investigated by quantum mechanical methods. “It’ll take a hundred years before that happens,” was his response before dashing off. This book is, in a sense, directed to that scientist. He and I are older now, and while I have learned that many things take longer than we expect, I would like him to consider that some things may take less long. While his estimate may well be right for a completely worked out solution to the problem of molecular excitability, there is no better time to begin working toward that goal than now.

This book refers to results condensed-state physicists have obtained in materials that exhibit structural and behavioral properties similar to those of membranes containing voltage-sensitive ion channels. I hope that this book, by bringing together molecular excitability and condensed state physics, will confirm that biology and physics are parts of the same world.

For this work I am indebted to many people. At UCLA, my professors Robert Finkelstein, David Saxon, Marcel Verzeano and Jean Bath stand out. James Swihart, my graduate adviser at the Indiana University Physics Department, taught me to sail the choppy seas of research; while in Europe, he discussed my thesis with Alan Hodgkin. Other influential professors at Indiana included Alfred Strickholm, Ludvik Bass (then a visiting professor from the University of Queensland, Australia) and

Walter J. Moore. Helpful during my postdoctoral work at the New York University Physics Department were Morris Shamos, Robert Rinaldi, Abraham Liboff and Charles Swenberg, as well as Rodolfo Llinas and Charles Nicholson at the New York University Medical Center. By convincing me that classical electrodiffusion is inadequate as a mathematical model of excitable membrane currents, Fred Dodge and James Cooley prodded me into looking for the reason for that inadequacy.

Harvey Fishman was my mentor and collaborator at the University of Texas Medical Branch in Galveston and the Marine Biological Laboratory at Woods Hole; he remains my friend. At Woods Hole I met and was inspired by Kenneth S. "Casey" Cole. At Texas Southern University, Floyd Banks, Sunday Fadulu, Debabrata Ghosh, Oscar Criner and Mahmoud Saleh were research collaborators. Discussions with Fred Cummings, Rita Guttman, Lee Moore, Tobias "Toby" Schwartz, Gabor Szabo, David Landowne, Malcolm Brodwick, Susan Hamilton, Arthur "Buzz" Brown, Richard "Spike" Horn, Tony Lacerda, Sidney Lang, Georg Zundel and others helped keep me focused.

Donald Chang was instrumental in turning my focus from the membrane to the channel. Ichiji Tasaki has been a friend and colleague. They, together with William J. Adelman Jr., collaborated with me in organizing a conference and editing a book on structure and function in excitable cells, a precursor to this volume.

Stewart Kurtz, Robert Newnham and other members of the Materials Research Laboratory of Pennsylvania State University provided valuable insights into ferroelectricity. I was fortunate in meeting Vladimir Fridkin, as our discussions have been fruitful. Vladimir Bystrov, my collaborator and friend, has applied his knowledge of physics and his boundless energy to research, writing, translating and organizing conferences. Hervé Duclohier invited me to his lab to put predictions of my channel model to an experimental test with his collaborators. Said Bendahhou and his colleagues extended the test from parts of channels to whole channels. Michael Green, a friend and colleague, and Fishman have read parts of this book and provided valuable criticism; any remaining errors are of course my own.

I thank the many scientists on whose work I have depended, both those I have cited and—with sincere apologies—those I have not. My special gratitude goes to the authors whose illustrations provide figures in this volume, as well as to the permissions staffs of publishing houses and the Copyright Clearance Center. Jane Richardson kindly provided me with an updated version of a figure. The librarians who supplied me with research materials, particularly at the Butt–Holdsworth Memorial Library in Kerrville, Texas, deserve special mention. I appreciate the skill, patience and thoroughness of Springer editors Peter Butler, Tanja van Gaans and André Tournois, and typesetters Bhawna Narang and Nidhi Waddon. My wife and intellectual companion, Alice Leuchtag, has been a constant source of support and encouragement throughout the writing of this book.

It is my hope that scientists will maintain an awareness of the outcomes of their research, applying science only to the building of a more just and peaceful world, in harmony with our planet.

H. R. L.

CHAPTER 1

EXPLORING EXCITABILITY

Voltage-sensitive ion channels are macromolecules that act as electrical components in the membranes of living organisms. While we know that these molecules carry out important physiological functions in many different types of cells, scientists first became aware of them in the study of the impulses that carry information along nerve and muscle fibers.

1. NERVE IMPULSES AND THE BRAIN

Our species, *Homo sapiens*, is unique among animals in its abilities to manipulate symbols, having developed languages and conceptualized space, time, matter, life, ethics and our place in the universe. These abilities are localized in the brain, about 1.4 kilograms of pink-gray organ. The complexity of the brain extends from macroscopic to microscopic—from its highly convoluted surface, through a labyrinth of lobes, tracts, nuclei and other anatomic structures, through a dense tissue of interconnected cells, through a rich mosaic of membranes, to the large molecules that make up those membranes and the membrane-spanning helical strands within them.

It is remarkable that, despite the vast differences in human behavior from even that of our closest primate relatives, the molecular structures in our brains differ only in minor details from those of other mammals. Even more remarkable is the fact that such seemingly primitive forms as bacteria possess complex molecules that are shedding light on the details of related molecules in our brains.

1.1. Molecular excitability

The human body, like the bodies of other living organisms, is a tumult of electrical activity. Just as an electrocardiogram shows us that the heart is a powerful generator of electric currents emanating from the coordinated action of its nerve and muscle cells, so an electroencephalogram demonstrates that the brain likewise generates electricity. The cells of the heart, brain and other organs produce electric currents in the form of transient ion flows across the membranes that cover them. The membranes are mosaic sheets of lipid and protein molecules. While the lipids form effective electrical

insulators, proteins of a particular class are capable of dispatching pulses of rapid ion conduction. These switchable protein macromolecules are called *ion channels*.[1]

Ion channels of one type, *ligand-gated ion channels*, recognize and react to specific molecules in their environment. When these ligand molecules attach, the ion channel changes its conformation and starts (or stops) conducting ions. Examples of ligand-gated ion channels include receptors for tastes and odors, and the macromolecules that receive elementary messages from other cells in the form of chemical messenger molecules. Among these we find hormones, such as thyroxine and insulin, and neurotransmitters, such as dopamine and acetylcholine.

Ion channels of another type switch their conductivity in response to a change of the voltage across the membrane. These channels make it possible for impulses to travel along nerve and muscle fibers; it is to these *voltage-sensitive ion channels* that this book is devoted. Hybrid channels exhibit both voltage and ligand sensitivity.

The problem of the way ion channels respond to changes in membrane voltage—the problem of *molecular excitability*—has not been solved, although much progress has been made in this direction. This book will report on the background, history and ongoing efforts being made toward a solution of this problem. We will approach the problem from different directions, concerning ourselves not only with recent results, but also with earlier data and concepts.

1.2. Point-to-point communication

To make large, multicellular organisms, evolution has had to solve the important problem of communication within the body. For stationary organisms such as plants, that problem was essentially solved by sending signal molecules, hormones, in the fluids that move up and down the body. Hormones also play an important part in communication within animals, but the amount and speed of the information that can be sent by this endocrine system is limited in specificity by the number of different hormones that can be synthesized and recognized, and in speed by the circulatory system that transports them. To generate a system of communication capable of controlling the muscles of the body, producing visual images and other sophisticated tasks in fast-moving organisms, the "blind watchmaker," evolution, had to do better.[2] A rapid point-to-point communication system was required.

The solution, which appeared early during the evolution of such invertebrates as jellyfish, was for certain specialized cells to grow fibers of great length and to send waves of electrical and chemical energy along them. These *nerve impulses* are complex examples of solitary waves. They travel along a vast network of nerve fibers, the nervous system. Data about the external environment and the status of the body are fed into this point-to-point communications network from sense receptors. In vertebrates, the sense data are processed into responses and memories in the central nervous system, which consists of the brain and the spinal cord. Their outputs signal muscles to contract by way of *neuromuscular junctions*, and stimulate the endocrine system by activating glands.

Nerve impulses are wonderful and mysterious. Every perceived sound, sight, smell and taste reaches our brains, and our consciousness, by nerve impulses. Every muscular movement, whether an eyeblink, a uterine contraction or a heartbeat, is

controlled by nerve impulses. Even the release of chemical messengers such as adrenaline or testosterone is stimulated by nerve impulses.

The nerve impulse is an integral part of what we mean by *cellular excitability*, the ability of living cells to respond to their environment. Nerve impulses are waves that move along *axons*, the long tubular fibers of neurons. One convenient way to study them is to record their electrical signatures, called *action potentials*. Action potentials can also be recorded from muscle, gland and other cells. Vast numbers of experiments on a great variety of animal and plant cells have been carried out by biological scientists to study the electrical responses of excitable membranes; see Figure 1.1.[3] It is only by information from such experiments that our ideas regarding the underlying basis of excitability can be tested.

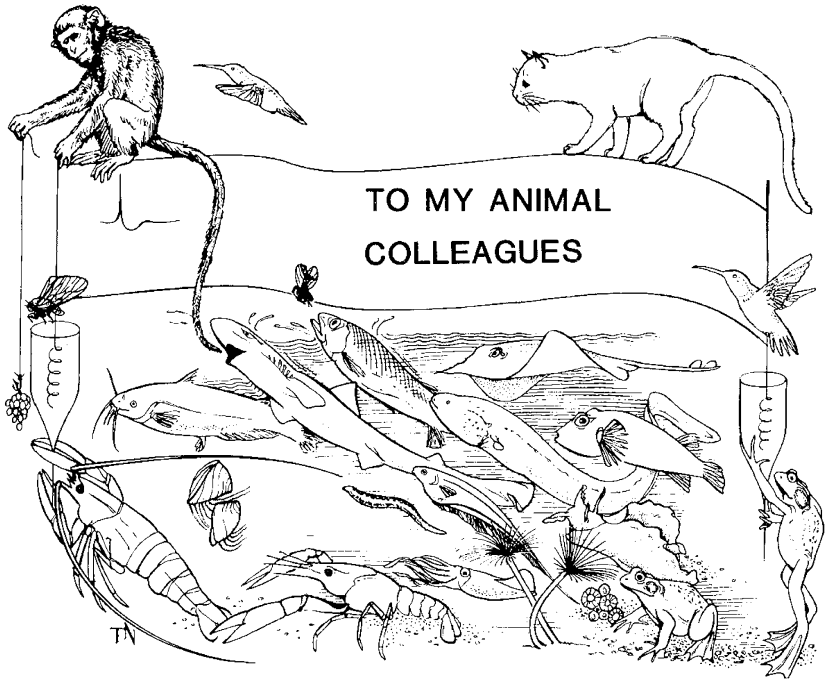

Figure 1.1. Dedication of a book on voltage-sensitive ion channels by Susumu Hagiwara. From Hagiwara, 1983.

1.3. Propagation of an impulse

The question *What is the scientific basis of excitability?* has intrigued scientists for centuries. Isaac Newton evidently had a strong interest in this question as he posed these two Queries in his book *Opticks*[4]:

> *Qu*. 23. Is not Vision perform'd chiefly by the Vibrations of [the Aether], excited in the bottom of the Eye by the Rays of Light, and propagated through the solid, pellucid and uniform Capillamenta of the optick Nerves into the place of Sensation? And is not Hearing perform'd by the Vibrations either of this or some other Medium, excited in the auditory Nerves by the Tremors of the Air, and propagated by the solid, pellucid and uniform Capillamenta of those Nerves into the place of Sensation? And so of the other Senses.
>
> *Qu*. 24. Is not Animal Motion perform'd by the Vibrations of this Medium, excited in the Brain by the power of the Will, and propagated from thence through the solid, pellucid and uniform Capillamenta of the Nerves into the Muscles, for contracting and dilating them? ...

Much has been learned since Newton's time (including the facts that the ether doesn't exist and that nerve impulses can make muscles contract but not dilate), yet the core of the question of excitability remains unresolved; a fundamental understanding of the molecular basis of the action potential still eludes us.

The neuron's long nerve fiber, the axon, is a long cylinder, which in some neurons of vertebrates has a fatty covering of *myelin* over it. The myelin sheath speeds up the action potential. For simplicity let us begin by considering an unmyelinated axon. The axon is bounded by a membrane called the *axolemma*, and it contains a watery, fibrous gel called the *axoplasm*. The axon is bathed in a body fluid that is essentially blood plasma, an aqueous solution rich in sodium ions, like seawater. The axoplasm has a much lower concentration of sodium ions, but a much higher concentration of potassium ions than the exterior solution. The anions, cations and neutral molecules present in the two solutions are distributed so as to make the solutions electrically neutral and at the same osmotic pressure. The sodium and potassium concentration differences represent two independent sources of energy.

A voltage measurement can tell us that a healthy axon, ready for a nerve impulse, is electrically charged. As a result of surface charges on the membrane, the axoplasm is negative relative to the external solution. The internal potential relative to the external solution in the inactive cell is called the *resting potential*. In a typical nerve axon, the potential of the axoplasm relative to the external medium (which serves as ground potential) is about -70 mV.

Sending a message requires a source of energy. Supplying energy only at the transmitting point would be inadequate, because the message would then diminish and be lost in the background noise. Energy sources therefore must be distributed all along the communication line. In this way, as in a line of carefully placed upright dominoes, there is no limit to the length of the path. However, a line of dominoes can send only one message—until energy is provided to set the dominoes up again. Thus for ongoing communication two sources of energy are needed: one to transmit the message (an impulse sufficient to knock the dominoes down) and another to restore the metastable order of the system (work to set them up again).

Such a strategy is used in the body to propagate nerve and muscle impulses. The nerve or muscle fiber is maintained in a high-energy state far from thermal equilibrium, known as the *resting* state. This term is a misnomer because the “resting” membrane is highly charged by a strong electric field. The resting voltage across a nerve membrane is usually about 70 mV, with the inside negative. Combining this with

the membrane thickness L of about 5 nm (1 nm = 10^{-9} m = 10 Å), we see that the average resting electric field, $E = V/L$, is of the order of 10^7 V/m, a very high field. So the "rest" of a resting membrane is a tense one indeed!

In this state, only a small stimulus is needed to initiate a wave in which the fiber rapidly falls to a state of lower energy. Part of the energy made available in this process must be passed along to a neighboring section to carry the wave on. This is the *fast system*, often called the *sodium system* for the current of sodium often ions involved in it. After the impulse has passed, the high-energy resting state—perhaps better called the *excitable state*—is restored to ready the system for the next impulse. This is accomplished by the *slow* or *delayed system*, often called the *potassium system*.

1.4. Sodium and potassium channels

The high concentration of sodium ions on the outside relative to that inside the cell would tend to drive them in. In addition, their positive charge attracts them toward the negative interior of the axon. For these two reasons, the external sodium ions are at a high *electrochemical potential energy* relative to the axoplasm, which would drive them across the membrane through any available pathway. Macromolecules called *sodium channels* within the axonal membrane provide such pathways under certain conditions. When these conditions are met, the channels are said to be *open*; otherwise, they are *closed*. The terms "open" and "closed" are convenient labels but, as we shall see in the following chapters, should not be taken too literally.

The voltage-sensitive sodium channels are pathways for sodium ions only when the membrane is *partially depolarized*. It takes only a rather modest depolarization (lowering of the absolute value of the voltage from resting) to reach the threshold at which the probability becomes high for the sodium channel to remodel itself into a different configuration, in which it becomes a selective ion conductor. Not all the sodium channels in an axon open to allow sodium ions to enter the axoplasm, however, and within a brief period of about 0.7 s most of them close again, even while the depolarization is maintained. Now the sodium system is said to be *inactivated*.

Restoring the excitable state—setting the dominoes upright again—is the job of the *potassium channels*. Like the sodium channels, these are glycoprotein molecules embedded in the fatty membrane. The probability that the axonal potassium channels will open increases upon depolarization, but only after a brief delay.

We emphasize an important point: The opening and closing of voltage-sensitive ion channels is not rigidly controlled by the membrane voltage. These are *stochastic* events, so that only their *probability* is voltage-dependent, as we will explore in Chapter 11.

1.5. The action potential

Now we can begin to see how a nerve impulse travels: Suppose a group of sodium channels in a region of an axon were to open. Then external sodium ions there would quickly enter the axon, pushed by the concentration difference and pulled by the

electrostatic force. As they carry their positive charges into the axoplasm, they drive the internal voltage toward zero and beyond, to positivity. As this *depolarization* spreads out within a local region surrounding the group of channels, neighboring Na channels sense it, respond and stochastically open, carrying the action forward. Like the line of dominoes, the array of sodium channels exists in a metastable situation; its destabilization spreads by local interactions. Thus the signal is carried from channel to channel and down the axon to its terminal. Because of inactivation, sodium channels close after a brief opening.

After a delay the potassium ions flow outward, driven by their electrochemical potential difference. Because the K^+ concentration is higher inside the cell, this current is oppositely directed to that of the sodium ions. The outward current restores the resting potential difference. It will take a little longer for that patch of axon to become excitable again; this *refractory period* is due to inactivation. The voltage-sensitive channels are restored to their excitable configurations by a shift of their molecular configurations, and the axon is ready to conduct another impulse.

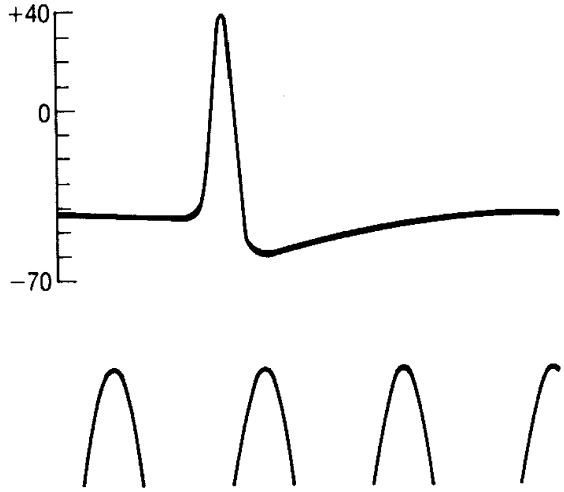

Figure 1.2. The action potential rises from the level of the resting potential to a positive peak, then drops at a slower rate to the resting potential. It may "undershoot" the resting level and approach it from below. The time marker, 500 Hz, shows that the action potential is complete in about 2 ms. This figure, published by Hodgkin and Huxley in 1939, is one of the first pictures of a complete action potential. From Smith, 1996. Reprinted by permission from MacMillan Publishers Ltd: Nature 144:710-711 copyright 1939.

An action potential, then, is a *traveling electric wave* normally initiated by a threshold depolarization, a sufficiently large lessening of the resting potential. (Alternately, it may be initiated by heating or injuring the axon or muscle fiber.) The entire action potential at a given point is completed in two to three milliseconds; it

propagates along the axon, which may be as short as a millimeter or as long, for example, as a giraffe's leg. Figure 1.2 shows the time course of an action potential.[5]

The action potential is not a localized phenomenon. As the inward current flows, the ions spread in both directions, depolarizing adjacent regions. This activates neighboring sodium channels, moving the impulse ahead. If the depolarization has been applied, experimentally, to an excitable region of an excised axon, the action potential may start off in either direction, depending on electrode placement. However, once the impulse has started, it will only continue in the forward direction, since the sodium channels in the backward direction are inactivated. In the living organism the anatomy of the cell ensures that the impulse travels in only one direction, from cell body to axon terminal.

We have seen that a useful way to look at a nerve axon is that it is a system of metastable units extended along a line, like a row of dominoes. The only thing that keeps the sodium ions from flowing in and the potassium ions from flowing out until electrical and diffusional equilibrium is attained is the impermeability of the membrane. Any breach in that impermeability will initiate an ion current. Evolution has found a way to harness that metastability by breaking the membrane's impermeability with two separate sets of molecules, permeable to different ions, thereby creating an efficient and adaptable system of information transfer. One type of ion channel is necessary to permit the signaling current to flow, and another to carry an opposing current to restore the membrane to its excitable condition. In many nerve and muscle membranes, the sodium channel plays the first, and the potassium channel the second role. This is not always the case; for example, calcium channels take the place of sodium channels at the axon terminal; the calcium ions they import into the cell trigger transmission of the signal across the synapse.

Necessary as well is the energy-requiring job of maintaining the different ion concentrations inside and outside the cell; this job is carried out by metabolically driven membrane molecules called *ion pumps*.

This brief (and incomplete) description shows us in general terms how an action potential works and what a voltage-sensitive ion channel does. What is missing from this simple picture is an understanding of the way the ion channels themselves work. That is the riddle of molecular excitability. Here begins the trail that we will seek to follow in this book.

1.6. What is a voltage-sensitive ion channel?

The electric currents that are measured in experiments on axons are due to the movement of positive ions across the axolemma. The major part of the membrane area is impermeable to ions; it is occupied by a double layer of lipid molecules. Lipids are amphiphilic molecules, arranged with their polar, hydrophilic heads facing outward to the aqueous phases. Because the inner regions of the membranes are composed of the hydrophobic tails, ions lack the energy to enter, much less traverse them. It is by way of the ion channels, glycoprotein molecules that extend through the lipid *bilayer*, that ions may, under certain conditions, pass. The relationship between the bilayer and the protein molecules intrinsically embedded within it has been explored by the

freeze–fracture technique; see Figure 1.3.[6] The carbohydrate chains of the glycoproteins are seen extending outward into the extracellular phase.

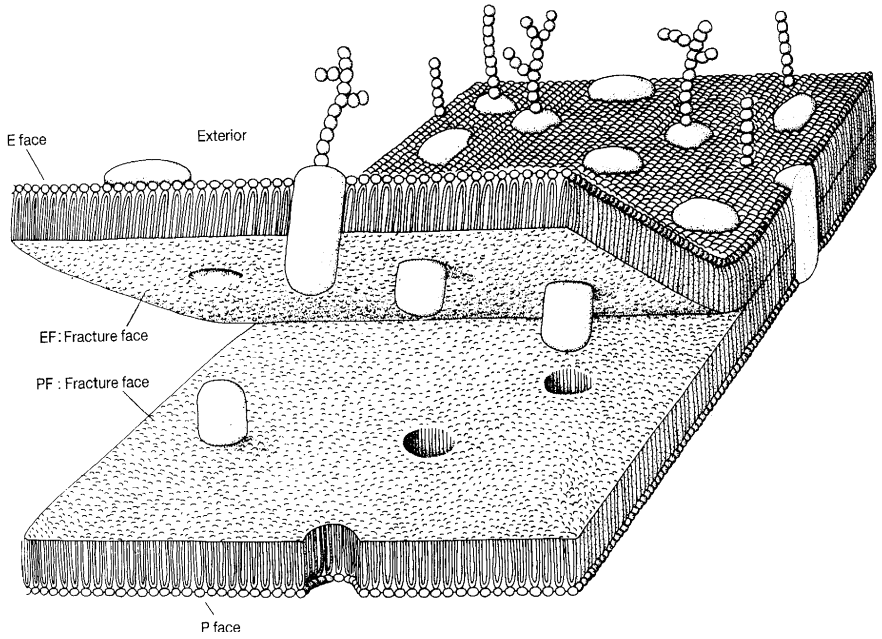

Figure 1.3. Schematic sketch of a cell membrane, showing the relation of intrinsic protein molecules to the lipid bilayer. From C. U. M. Smith, 1996, after B. Safir, 1975.

Among the various types of membrane proteins we shall focus on the ones directly involved in excitability. We have already mentioned the sodium channel and the calcium channel, rapidly switching conductors of their respective ions, and the slower potassium channel. These glycoprotein molecules are called *voltage-sensitive* (or voltage-dependent)[7] ion channels, because it is the voltage across the membrane that controls their ion conductance. Because the ion concentrations inside the axon are different from those outside, the concentration differences act, along with the potential difference, to move the ions. The voltage plays two roles: Its decrease impels a change in the conformation of the molecules in their ionic environment, and it helps to drive the ions across.

In later chapters of this book we will review how these channels behave in various circumstances, that is, their *function*, and how they are put together, their *structure*. We will seek to answer the questions:

- How do the ions pass so rapidly through the voltage-sensitive ion channel?
- How does the channel manage to select specific types of ions to carry?
- What transformations does the conformation of the channel undergo that

convert it from nonconducting to conducting and back?
- How are the opening and closing transformations coupled to the electric field?
- How does the structure of channels determine their function?

These are difficult questions and, although various models have been proposed, the full answers to them are not yet known. We can expect the answers to be rather subtle, and that it will require a great deal of fundamental knowledge to understand them. For this reason let us now take a brief tour through some aspects of the sciences of physics, chemistry and biology and their interdisciplinary combinations.

2. SEAMLESS NATURE, FRAGMENTED SCIENCE

One of the fundamental tenets of science is that nature is a seamless unity. Yet a survey of science as it is actually carried on shows that, in practice, science is divided into disciplines represented by departments with little communication between them. This division into physics, chemistry, biology and other branches, historically necessary though it was, has resulted in a fragmented science.

2.1. Physics

Physics is a set of general concepts that deal with such concepts as space, time, force, motion, electricity, magnetism, sound, light and the fundamental structure of matter. These concepts are as important to living as to nonliving things, to "the trees and the stones and the fish in the tide."[8]

Newton's mechanics is the flagship theory of classical physics. *Classical mechanics* allows us to isolate a problem from its environment. Newton's three laws are sufficient for many applications but fail in two realms: the fast-moving and the microscopic. The two revolutions that dealt with these realms are relativity and quantum mechanics.

In solving a mechanical problem, the direct application of Newton's laws is usually *not* the easiest way to proceed. Instead of analyzing forces, the concept of *energy* gives us a more convenient approach, because of the important law that energy is conserved. The concept of energy conservation extends far beyond mechanics, because energy takes many forms, including heat, electrical, magnetic, elastic and chemical—even mass, as relativity shows, is a form of energy. Energy is not necessarily associated only with particles, but can be found in space, in the form of *fields*—electric, magnetic and gravitational.

One branch of physics directly pertinent to voltage-sensitive ion channels is *electrodynamics,* which deals with electricity and magnetism. While mechanics describes a world of three independent dimensions, length, time and mass, nature provides another dimension: electric charge. This dimension adds some interesting phenomena: Resting charges produce electrostatic attractions and repulsions; when charges move, they also produce magnetic fields, perpendicular to the velocity or current. Electric and magnetic fields in space produce electromagnetic waves. Thus