1 Introduction

The book to read is not the one that thinks for you, but the one which makes you think.

— James McCosh (1811–1894)

1.1 Why Study Biophysics?

Why should a young neuroscientist study cellular biophysics and modeling? One answer is that the electrical properties of cell plasma membrane and signal transduction are essential to cellular and systems neuroscience. Another answer is that the biophysics of individual neurons is a well-understood subject. The experimental electrophysiology and computational modeling performed over the last century has led to scientific consensus on the "right way" of thinking about electrical and chemical signaling of neurons and the collective activity of small neuronal networks. There is no such consensus on central nervous system function.

> Ask 10 of the world's leading neuroscientists how the brain works – how it thinks, feels, perceives, and acts as a unified whole – and you will get 10 different answers, unless they are very narrowly framed around the biophysics and chemistry of nerve impulse conduction and synaptic transmission (Swanson, 2012).

Admittedly, cellular biophysics may seem to be a narrowly framed subject, especially if you are an undergraduate who is primarily interested in behavioral or cognitive neuroscience. On the other hand, the electrical and chemical signaling of neurons – the elementary anatomical units of the central nervous system – is absolutely essential to brain function. Cellular biophysics and modeling is foundational to cellular and systems neuroscience and a solid point of departure to more comprehensive study of brain, mind and behavior.

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1.2 Neurons are Brain Cells

It goes without saying that neurons are brain cells – elementary anatomical units of the central nervous system that are polarized to mediate input/output functions: dendrites → soma → axon → synapses. Rinse, repeat. The scientific history of these foundational convictions is the subject of a book by Gordon Shepherd entitled *Foundations of the Neuron Doctrine*. The cellular structure of the central nervous system was first observed in sections of neural tissue using staining methods developed by Camillo Golgi and later perfected by Santiago Ramón y Cajal. Because a small proportion of neurons were labelled, the structure of single neurons could be resolved (see Fig. 1.1). Golgi's observations reinforced his working hypothesis that neural tissue had a reticular structure analogous to the circulatory system. Cajal concluded that each neuron was a separate entity that interacted with other neurons at synaptic junctions. The 1906 Nobel Prize in Physiology or Medicine was awarded jointly to Golgi and Cajal in recognition of their work on the structure of the nervous system.

More than a century later, neurons (and glia) are still brain cells. However, some aspects of the neuron doctrine are difficult to sustain. Today's neuroscience students

> . . . could well conclude that the nerve cell is not the unit of function that is of primary interest to them. The units of contemporary studies are the packets of transmitter molecules, the channels and receptors by means of which nerve cells communicate with each other. Although many

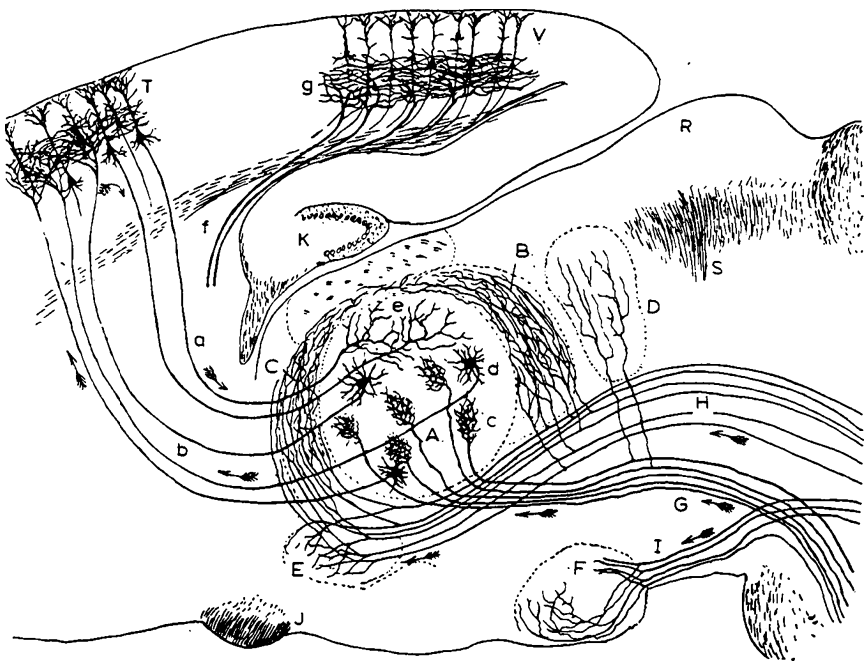


Figure 1.1 A sagittal section through the rat brain as drawn by Ramón y Cajal. The section shows thalamocortical neurons that project to cortex (d and b), and cortical pyramidal neurons that project to the thalamus (T, a, e), and much more. Reproduced from Cajal's 1906 Nobel lecture *The structure and connexions of neurons*. © The Nobel Foundation 1906.
Question: Where is the hippocampus?

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nerve cells are polarized in the classical sense, others are not: parts of the classical neuronal output system, the axons, can serve as receptors, and the classical receptor portions of the dendrites can serve as effectors. . . . There are many examples of nerve cells linked to each other by specialized gap junctions that provide electrical coupling and allow the passage of small molecules from one nerve cell to another. . . . There are nerve fibres that are produced by the fusion of processes from several cells. . . . These facts are all contrary to the neuron doctrine, as originally expressed (Guillery, 2005).

Discuss Neurons are discrete anatomical components of the central nervous system, but does this imply that neurons are the elementary physiological units subserving the computations performed by the brain?[1]

1.3 Cellular Biophysics

Biophysics is a thriving scientific discipline, but it can be difficult to define. In *Biophysical Journal*, Olaf S. Andersen recounts that the centerpiece of biophysical research

. . . in the early part of the twentieth century was neuro- and muscle physiology, disciplines that lend themselves to quantitative analysis and in which most of the investigators had trained in biology or medicine. In the latter half of the century, an increasing number of biophysicists were trained in chemistry, physics, or mathematics, which led to the development of the modern generation of optical and electron microscopes, fluorescent probes . . . as well as the computational methods that, by now, have become indispensable tools in biophysical research (Andersen, 2016).

What is biophysics? Archibald Vivian Hill[2] emphasized that physical instrumentation in a biological laboratory does not a biophysicist make. Rather, it is the biophysical *mindset* that is important. Biophysicists attempt to understand biological structure, organization and function using the ideas and methods of physics and physical chemistry (Hill, 1956).

I use *cellular biophysics* as a flexible term for quantitative, physical and physicochemical approaches to the complex phenomena of cell biology and neuroscience. These include, but are not limited to, the electrical properties of the plasma membrane of neurons (e.g., voltage- and ligand-gated ion channels), cell signal transduction (e.g., ionotropic and metabotropic receptors, intracellular calcium responses), and aspects of biochemistry and cell biology (e.g., metabolic oscillations, microtubule dynamics and cell motility).

Cellular biophysics is somewhat, but not entirely, distinct from *molecular* biophysics, e.g., the use of nuclear magnetic resonance (NMR) to determine the structure of macromolecules. Both subjects are inherently interdisciplinary and highly dependent upon physical techniques. The experimental methods specifically relevant to cellular biophysics include, but are not limited to, voltage-clamp electrical recordings, confocal microfluorimetry, and fluorescence resonance energy transfer (FRET).

A wide variety of small molecules contribute to the electrical and chemical signaling of excitable cells such as neurons and myocytes. Examples include: neurotransmitters (glutamate, glycine, gamma-aminobutyric acid = GABA), hormones (epinephrine = adrenaline, vasopressin, cortisol, estrogen), lipids (PIP_2 = phosphatidylinositol 4,5-bisphosphate, ceramide, sphingosine) and second-messengers

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(cyclic adenosine monophosphate = cAMP, inositol trisphosphate = IP_3, diacylglycerol = DAG, calcium = Ca^{2+}).

The macromolecules that dominate cellular biophysics are the integral and peripheral membrane proteins involved in cell signaling and membrane transport. Integral membrane proteins are permanently attached to the cell plasma membrane (e.g., cell surface receptors) or intracellular membranes. Integral membrane proteins of the cell plasma membrane often span the lipid bilayer and have significant extracellular and intracellular (as well as transmembrane) domains. Peripheral membrane proteins attach (sometimes fleetingly) to integral membrane proteins or the inner leaflet of the lipid bilayer (e.g., phospholipase C, which hydrolyzes PIP_2 into the second messengers IP_3 and DAG).

Fig. 1.2 summarizes the classes of transmembrane proteins that are most relevant to our study of cellular biophysics. These include neurotransmitter receptors, ion channels, and transporters (e.g., pumps and exchangers). An ionic channel is a membrane protein with an aqueous pore; when the channel is permissive, certain ions may pass through the pore, crossing from one side of the cell plasma membrane to the other. Examples include non-gated potassium "leak" channels, potassium channels that are gated by the binding of cytosolic calcium, voltage-gated sodium channels, and

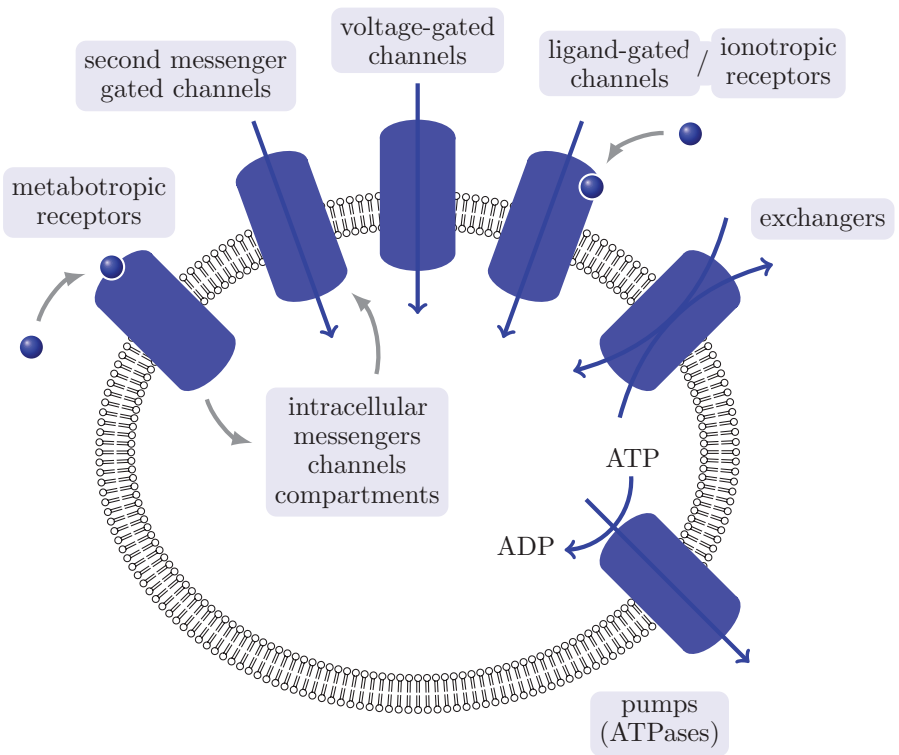


Figure 1.2 Integral membrane proteins involved in membrane transport include neurotransmitter receptors, ion channels and transporters (exchangers and pumps).

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glutamate receptors (ligand-gated ion channels permeable to both sodium and potassium). Pumps are transporters that couple the hydrolysis of ATP to the movement of one or more ions against concentration or electrochemical gradients. Examples include the sodium-potassium ATPase and the sarco-endoplasmic reticulum calcium ATPase (SERCA).[3] Exchangers are transporters that use the concentration gradient of one type of ion to catalyze the movement of another (e.g., the sodium-calcium exchanger).

This primer does not cover the molecular and structural biology of ion channels, transporters and pumps. Rather, our emphasis is the *functional role* of ionic channels in the electrophysiology of neurons, myocytes and other excitable cells. Because the physiological states of living cells measured using biophysical techniques are complicated functions of time, our study necessarily involves the mathematics of dynamical systems.

Discuss What are some examples of physiological signals (electrical or chemical) that are routinely measured by physicians and experimental biologists? What is the role of these physiological measurements in medical diagnosis?

1.4 Dynamical Systems Modeling

In mathematics, a dynamical system is a rule that describes how the location of a point in geometrical space changes as a function of time. When this concept is used to model dynamical phenomena of cellular biophysics, the "point" is some aspect of the state of a living cell (e.g., the membrane potential or the concentration of a chemical species) in the "space" of all realizable states (e.g., a concentration may not be negative). While the rule for the time-evolution of a dynamical system can take many different forms, a natural choice is to specify the rule using one or more differential equations.

Differential equations are used by engineers and physicists to describe and predict change in the physical world, that is, the time-dependent dynamics of inanimate objects. In physics (classical mechanics) Newton's second law of motion describes the relationship between the net force acting on a body[4] and its motion using the familiar equation, $F = ma$, where $F(t)$ is the force applied, m is the mass of the body (constant), and $a(t)$ is the body's acceleration. Acceleration is rate of change of velocity, that is, $a = dv/dt$; and momentum (p) is mass times velocity $p = mv$. Consequently, Newton's second law can be rewritten as a differential equation[5] that describes how the body's momentum changes in response to a force, $dp/dt = F(t)$. From the dynamical systems perspective, this differential equation is a rule that determines how the momentum $p(t)$ (the state of the system) changes in response to the time-dependent force $F(t)$.

In a similar manner, many contemporary biologists apply dynamical systems to the study of life (e.g., ecologists model population dynamics of food webs, and public health experts model epidemics of communicable disease). Just as chemical engineers use differential equations to describe and predict the kinetics of chemical reactions, biochemists use differential equations to analyze and simulate metabolic pathways. Modeling with differential equations is paramount in systems physiology and pharmacokinetics. The approach has long been employed to study the flow, between different organs in the body, of dissolved gases, nutrients, drugs, hormones and radio-isotopes.

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When it comes to the physiology of excitable cells – those cell types that can generate action potentials in response to depolarization, such as neurons and cardiac myocytes – the dynamical systems perspective has been with us for over half a century. Alan Hodgkin and Andrew Huxley's Nobel Prize winning studies[6] of the action potential in the squid giant axon included dynamical systems modeling with differential equations. Today, Hodgkin-Huxley-style modeling is the dominant framework for studying and analyzing ion channel kinetics and the physiological consequences of differentially expressed ionic currents in neurons and other excitable cells (e.g., myocytes, pancreatic beta cells, saccular hair cells). The dynamical systems perspective is a key ingredient of a historically accurate and scientifically rigorous understanding of the physiology of excitable cells.

Discuss Putting dynamics and differential equations to one side, what other types of mathematics are important to the life sciences?

1.5 Benefits and Limitations of Mathematical Models

Life scientists study experimental model systems such as nematodes (roundworms) and drosophila (fruit flies) because this practice facilitates scientific discovery. Experimental model systems are developed and utilized because they lend themselves to investigation (e.g., due to a technical or ethical advantage). Similarly, mathematical models in the life sciences are idealizations of biological phenomena that have certain advantages. Like experimental model systems, mathematical models also have limitations.

This book emphasizes how a conceptual model – e.g., a verbal description or a cartoon summary of a hypothesis – can be converted into a dynamical systems model composed of one or more differential equations (Fig. 1.3). When this process of bringing cartoons to life[7] is mastered, one learns that mathematical models are natural manifestations of conceptual models, but with significant advantages. While the implications of a conceptual model are often unclear, mathematical models may be analyzed by hand calculations, graphical techniques and computer simulation to clarify the implications of the parent conceptual model. As we will see, conclusions often depend on model parameters (e.g., rate constants) or aspects of the conceptual model that were indeterminate or obscure. In this way, mathematical modeling often sharpens scientific hypotheses by highlighting the subtleties hidden within conceptual models.

The limitations of mathematical models are obvious to experimental scientists and are often emphasized. We are often reminded that a mathematical model is an abstraction and not a biological reality. Of course, this point should always be kept in mind, just as we should never forget that conceptual models are also abstractions!

The idealization that occurs in biological modeling is an advantage as well as a limitation. Unlike biological reality, a mathematical object can in principle be completely understood. Theorists and experimentalists agree that differential equations are usually easier to interrogate than the corresponding tangible systems that they represent. The analysis of mathematical models often results in qualitative "take home messages" that are heeded by experimental scientists.

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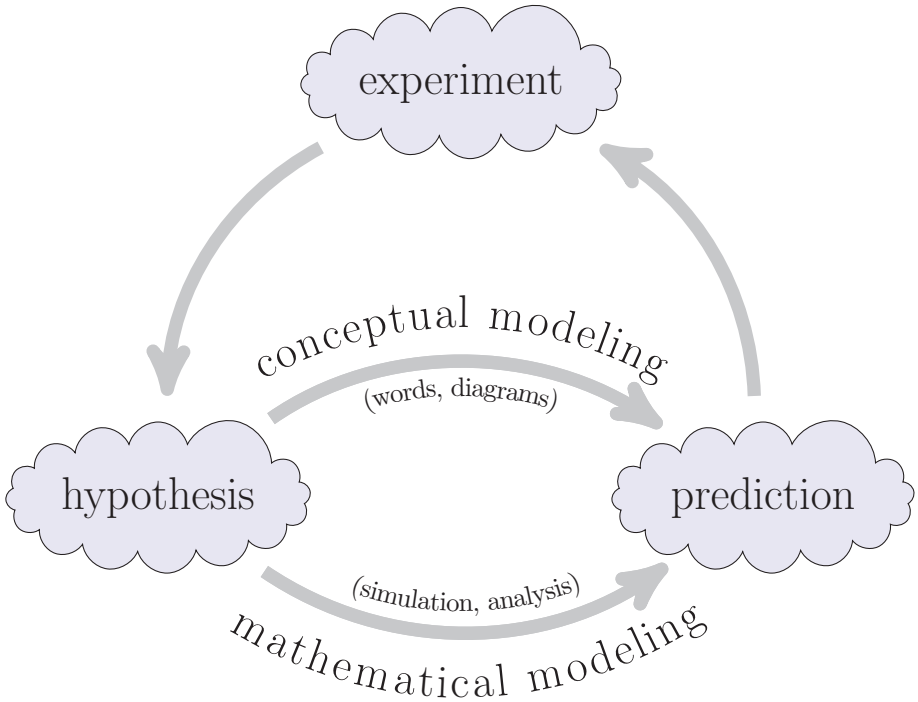


Figure 1.3 The cycle of experiment, hypothesis and prediction in the biological sciences. Moving from hypothesis to prediction requires either conceptual or mathematical modeling.

Discuss Mendel's laws are fundamental to our understanding of the genetics of inheritance. What aspects of Mendel's laws are idealizations of biological reality? Is Mendelian genetics an example of conceptual modeling or mathematical modeling? How about the Hardy-Weinberg model?

1.6 Minimal Models and Graphical Methods

Mathematical models come in many varieties and are constructed for different purposes. Highly realistic mathematical models of living cells can be so extremely complex that detailed analysis is time consuming and impractical even with the assistance of sophisticated software packages and high-performance computers. Although physiological realism is an important goal, computational models that are nearly as difficult to understand as the corresponding experimental system are not particularly useful.

To see this, imagine a pedestrian tourist who requires a map to help explore a city. A good map has a clear correspondence to the city, but this agreement is reduced, abstract and approximate, because the map must be easily carried and read. A perfectly realistic map with exact correspondence to the city would be the *size* of a city and completely useless, because reading the map would be no less difficult than exploring the city on foot.

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In the same way, a helpful mathematical model is physiologically realistic to some degree, but not so complex that it cannot be analyzed and well understood. A mathematical model should be simple enough to be comprehended by the theoretician and realistic enough that this understanding is relevant to the experimentalist. Mathematical models that display this balance between realism and complexity are often referred to as **minimal models**. A mathematical model that is understood to be oversimple but is nevertheless of interest is a **toy model**.

One of the benefits of minimal models is that they can be analyzed, sometimes through pencil and paper calculations, using the qualitative theory of dynamical systems. This geometrical approach to analyzing differential equations is highly visual, easy to learn, and produces intuition about dynamical systems that is of benefit to the life scientist. Learning this graphical way of thinking about mathematical models is well worth the effort, even if one does not intend to pursue mathematics further, because these tools allow one to quickly glean the ambiguities and take home messages of conceptual models. With the help of special purpose software packages, these graphical techniques can also be used to analyze complex mathematical models.

Discuss The phrase "Everything should be made as simple as possible, but not simpler" is often attributed to the theoretical physicist Albert Einstein. What exactly did he mean by this? How is this maxim relevant to mathematical modeling in biology?

1.7 Biophysics and Dynamics Together

Cellular biophysics and differential equations are both challenging subjects. Teaching both simultaneously might seem like an ill-fated pedagogical choice. Attempting to learn both subjects at the same time might feel like a daunting task. However, there are benefits to communicating mathematical and biological ideas in an integrated manner (Fig. 1.4).

First, teaching dynamics and biophysics at the same time shows students how mathematical modeling is actually used in cell physiology and neuroscience. Golgi and Cajal did not use dynamics in their investigations of the microscopic structure

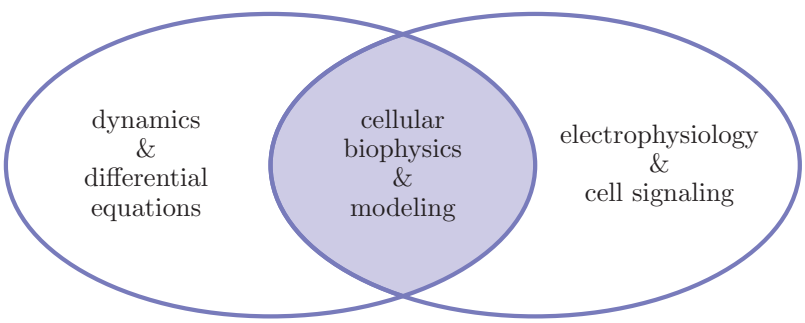


Figure 1.4 *Cellular Biophysics and Modeling* is an exploration of the physiology of excitable cells and the mathematical language of dynamics (differential equations).

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of the brain. But differential equations were used by Alan Hodgkin and Andrew Huxley to explain the initiation and propagation of action potentials in the squid giant axon. Contemporary cellular and systems neuroscience is highly quantitative, and the cognitive and behavioral neurosciences are becoming more quantitative with each passing year.

Second, mathematical modeling is best learned through the experience of many specific, concrete examples that are relevant to one's scientific interests. For biology and neuroscience students learning about membrane excitability and cell signaling, this means that physiological examples and exercises are preferred. Although we mentioned Newtonian mechanics above, you will not find any oscillating pendula in this book! This leads us to the third, and perhaps most important, benefit of learning dynamics and electrophysiology at the same time: deeper understanding.

We choose to teach cellular biophysics and differential equations in an integrated manner because, frankly, the mathematical perspective is required to achieve a deep understanding of the dynamical phenomena that comprise electrophysiology and cell signaling. The electrical properties of individual neurons and myocytes and, by extension, the function of the central nervous system cannot be understood without the language of mathematics. The possibility that one may be teaching undergraduate neuroscience majors does not change this. In the words of Bob Marley, we must "tell the children the truth."

1.8 Discussion

Students new to neuroscience will benefit from reading the classic *Scientific American* article "The organization of the brain" (Nauta and Feirtag, 1979) and the introductory chapters of Swanson (2012) and Schneider (2014).

For further reading on the neuron doctrine see the monograph Shepherd (2015) as well as articles by Glickstein (2006), Bullock et al. (2005) and Guillery (2005). The Nobel lectures of Cajal (1906) and Golgi (1906) are available at `www.nobelprize.org`.

The history of theory in several areas of biology is discussed by Shou et al. (2015). The short *Nature* essay "Bringing cartoons to life" by Tyson (2007) argues that because cells are dynamical systems, mathematical tools are required to understand the relationship between molecular interactions and physiological consequences.

My answer to "What is biophysics" draws heavily from Andersen (2016). The Biophysical Society web page is `www.biophysics.org`.

Cells and Computers

In his lectures on mathematical modeling of the cell cycle, computational cell biologist John Tyson asks students to consider the similarities and differences between an individual cell and a computer. Computers and cells are different in that computers are manufactured while cells are self-reproducing, but similar in that both obey the laws of thermodynamics. Computers process an input and produce an output. Do cells function in this manner? If so, how are the processing steps occurring within cells and computers similar or different?

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How Many Neurons?

The number of neurons in the human central nervous system is estimated to be somewhere between ten billion (10^{10}) and one trillion (10^{12}) (Nauta and Feirtag, 1979). Truly, these are astronomical numbers, as there are thought to be 200–400 billion stars in the Milky Way galaxy. Imagine a neuron represented by a coarse grain of sand with linear dimension of half a millimeter. The volume of a cubical grain of sand would be

$$(0.5\,\mathrm{mm})^3 = (0.5 \times 10^{-3}\,\mathrm{m})^3 = 1.25 \times 10^{-10}\,\mathrm{m}^3\,.$$

A sand castle representing a human brain composed of 10^{10} grains of sand (lower estimate) would have a volume of

$$(10^{10})(1.25 \times 10^{-10}\,\mathrm{m}^3) = 1.25\,\mathrm{m}^3\,,$$

that is, about a cubic meter. Although it is not difficult to visualize this amount of sand, placing the grains side by side in linear array would yield a row fifty thousand kilometers long, which is greater than the circumference of the earth.[8]

Problem 1.1 The upper estimate of 10^{12} neurons per brain is 100-fold greater than the lower estimate. Repeat the above calculation using this value. What is the fold increase in the height of the cubical sandcastle?

Problem 1.2 Estimate the number of neurons in a cubical human brain under the assumption that a neuron may be represented by a cube that is 10 micrometers on a side.

Know Your Neurons

Cajal's drawing of a sagittal section of a rat brain includes thalamocortical and cortical pyramidal neurons (Fig. 1.1). Are these neuron types also present in the human brain? How many morphological types of neurons can you recall and/or visually identify after a Google image search?

Is Neuroscience Hard or Soft?

In the so-called hard sciences (e.g., physics), there are a small number of causative variables, the language of mathematics formalizes hypotheses, and predictions of hypotheses can be rigorously derived. In the soft sciences (e.g., psychology), numerous variables are in play, most of which are difficult to quantify. In the soft sciences, hypotheses are often not formalized using mathematics, the predictions of hypotheses are debatable, and empirical observations rarely lead to strong conclusions. On the other hand, one could argue that the soft sciences are in some ways harder (more difficult) than the hard sciences, because the phenomena addressed are more complex and less easily understood. Is neuroscience a soft science or hard science? Why might some scientists and historians of science find this soft/hard terminology problematic?

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Of Math and Majors

To what degree does mathematics play an important role in the science and social science majors at your college or university? Does your answer correlate with the reputation of each subject for rigor and difficulty? To what extent did mathematics play a role in your choice of major?

The Effectiveness of Mathematics

When scientists drill down into physical science, the black gold of mathematics is discovered again and again. In fact, the relationship between the empirical and (applied) formal sciences is so tight, and experienced so routinely by mathematical scientists, that we tend to lose sight of the wonder of this connection. Why does mathematics play an essential role in physical theory?

Solutions

Figure 1.1 The hippocampus is labelled with a K.

Problem 1.1 The fold increase in height is $10^{2/3} \approx 4.64$.

Problem 1.2 An estimate of the number of neurons per brain would be given by the ratio of the brain volume and the neuron volume,

$$\frac{\text{brain volume}}{\text{cell volume}} = \frac{(10\,\text{cm})^3}{(10\,\mu\text{m})^3} = \frac{(0.1\,\text{m})^3}{(10^{-5}\,\text{m})^3} = \frac{10^{-3}\,\text{m}^3}{10^{-15}\,\text{m}^3} = 10^{12}\,,$$

that is, one trillion, consistent with the upper estimate in Nauta and Feirtag (1979).

Notes

1. Complex synaptic structures known as glomeruli suggest that subcellular regions of a neuron can operate as distinct functional units (Sherman and Guillery, 2004).
2. The English physiologist who shared the 1922 Nobel Prize in Physiology or Medicine for his discovery "relating to the production of heat in the muscle."
3. The organelle known as the endoplasmic reticulum (ER) in neurons and many other cell types is, among other things, an intracellular calcium store. Sarcoplasmic reticulum (SR) is a specialized version of ER that occurs in skeletal and cardiac muscle cells.
4. For simplicity, F, a and p are assumed to be scalars as opposed to vectors. We are ignoring rotation and assuming a rigid body constrained to move in one dimension. $F(t)$ is a force applied to the body's center of mass.
5. The equation $dp/dt = F(t)$ is an example of an *ordinary* differential equation (ODE) as opposed to a *partial* differential equation (PDE).

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6. Together with John Carew Eccles (1903–1997), Andrew Fielding Huxley (1917–2012) and Alan Lloyd Hodgkin (1914–1998) won the 1963 Nobel Prize in Physiology or Medicine "for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane." For historical perspective see Schwiening (2012).
7. "Bringing cartoons to life" is the title of a highly recommended *Nature* essay by John Tyson (2007).
8. The circumference of the earth is about 40,000 kilometers ($4 \times 10^7\,\mathsf{m}$). The row of sand grains would be at least $10^{10} \cdot 0.5\,\mathsf{mm} = 5 \times 10^7\,\mathsf{m}$ in length.

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