

# Chapter 12

## Computer Simulations of Basic Neuronal Function

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## Contents

Introduction.....	156
Materials .....	156
Notes for the Instructor .....	156
Student Outline .....	157
Acknowledgements.....	162
Literature Cited.....	162
Appendices.....	163

## Introduction

Computer simulations can be useful tools in the teaching of many physiological functions. The programs have the advantage of being cost-effective in the long term, as well as eliminating the sacrifice of experimental animals. In some cases, such as in the *Neurosim* software used in this exercise, students are able to carry out more experimental manipulations than if *in vivo* studies were being utilized.

*Neurosim* can be used for first-year students being introduced to basic neuronal function or for advanced students in neurophysiology. Since the four inclusive programs within the software package operate independently instructors can utilize only certain aspects of the program and thus customize it to meet the needs of their course. As well, the ease of operating the programs makes students much less hesitant to experiment with many aspects of the software, something they might not have done had they been using a live preparation. Following up the computer simulation exercise with a discussion of the underlying principles of the programs allows for instant feedback for students to see if they have grasped some of the difficult aspects of neurophysiology.

## Materials

IBM-compatible computer equipped with CGA or EGA color graphics card  
*Neurosim* computer software from BIOSOFT, 22 Hills Rd., Cambridge, U.K. CB2 1J  
 LCD panel with DOS cables and appropriate overhead (*optional*)

## Notes for the Instructor

*Neurosim* is a software package designed for use in undergraduate or graduate physiology labs. There are four programs, each of which operates independently. CABLE.EXE and HH.EXE are covered by this exercise and are suitable for undergraduate laboratories. PSP.EXE (Post-synaptic Potentials) and NETWORK.EXE (Neural Network) are more suitable for advanced classes.

The manual by Heitler (1988) that accompanies the software states “that the programs are intended to be run on IBM-PC, -XT, -AT compatible computers equipped with either the Color Graphics Adaptor (CGA) or the Enhanced Graphics Adaptor (EGA) or true compatibles. The EGA should have the full complement of 256 kilobytes of display memory. The computer must have at least one floppy disk drive. A 'snapshot' of the screen can be printed at any time if an IBM/Epson graphics-compatible printer is connected to the standard print port.” It is written in a straightforward and easy-to-read manner. The manual is divided into three parts. The first section gives a general description of the programs. The second section concentrates on each of the individual programs, discussing the parameters to be simulated and the range of numbers to chosen. The third section gives helpful examples of the four programs as well as a tutorial section.

All the programs are user friendly. While a thorough knowledge of nerve physiology is important to the understanding of the underlying principles exemplified by this software (see Eckert et al., 1988; Kandel et al., 1991), no previous knowledge of the software use or disk operating system is necessary for the successful completion of this exercise. On-line help is available throughout each step of the programs with a detailed explanation of the parameters listed for the simulation and what steps should be used to continue or end the program.

While not essential to the success of this lab, the use of a LCD panel with appropriate overhead connections to one computer will allow for easier explanation of the formal steps of this lab and will facilitate the student's understanding of the operating procedures.

Allow considerable lead time when writing to BIOSOFT as we have had long delays in getting replies from the company. When we purchased our software in 1991 the cost was \$600 CDN for the four programs.

We use HH.EXE as a demonstration in our introductory course to show the basic characteristic of an action potential. In our full-year physiology course we use the exercise as described here along with a discussion session. *Neurosim* is one of four neurophysiology-related labs that we do. Prior to *Neurosim* we have a membrane transport lab using artificial membranes and a Ussing Chamber. After the *Neurosim* exercise we have the students do a lab on the neuromuscular junction in the lobster walking leg and a lab on the mechanoreceptor organ in the crayfish. This package of four labs has been very successful for our students.

## Student Outline

### Introduction

The classic laboratory exercise normally used to examine basic neurophysiological principles is the generation and conduction of compound action potentials in the isolated frog sciatic nerve. While this has been a particularly effective lab in its reliability, its limited scope of demonstrable concepts, combined with the declining populations of *Rana pipiens*, has necessitated a change to a more feasible lab.

For several years, computer programs have been available that model the electrical behavior of non-excitabile and excitable cells. Such programs were initially developed so that biologists could easily test and modify the mathematical models which have been developed to explain the electrical activity of neurons. By comparing the electrophysiological behavior of the computer-simulated model neuron to real neurons studied via conventional electrophysiological techniques, experimenters derive a better understanding of the validity of assumptions and treatment of variables that are inherent in their mathematical models. Early computer tests of the model describing nerve excitation (i.e., Hodgkin-Huxley Model) are the most classic examples of such an approach.

For the same reasons that computer models are beneficial to the basic scientist, they can be extremely helpful to the student of electrophysiology. These programs allow the student to create their own experimental neurons by "programming in" values to a variety of variables that are important in determining electrical activity. For example, the student can change the bath concentration of  $\text{Na}^+$  to a higher value and then record what effect this might have on the action potential amplitude. The result generated by the computer is in the form of an electrophysiological recording, exactly as would occur in the actual electrophysiology lab if it were displayed across an oscilloscope screen. The computer program that will be used in this lab not only allows the experimenter to try experimental manipulations that parallel those done in a real electrophysiological experiment (see Hodgkin and Rushton, 1946; Hodgkin and Huxley, 1952), it also allows one to change some of the inherent properties of neurons, such as membrane resistance,

axon diameter, etc. This enables one to see how neurons of different physical characteristics differ in their electrical behavior.

Two computer programs will be used in today's lab, all written as part of a package called *Neurosim* (BIOSOFT, Cambridge, U.K.). CABLE.EXE simulates passive conduction in a long length of axon. HH.EXE simulates the Hodgkin-Huxley equations for the production of an action potential.

### Materials and Methods

IBM-compatible computer equipped with CGA or EGA color graphics card  
*Neurosim* computer software from BIOSOFT (Cambridge, U.K.)

Since you are not expected to learn the disc operating system for the purposes of this lab, the instructor will instruct you individually how to gain entry into the desired program. Your instructor should access the *Neurosim* directory for you. The prompt command **c:\Neurosim** should be on the screen when you begin. Simply typing **<START.BAT>** should get it started, as long as you are in the correct directory. Please have the instructor show you this procedure, as it may be slightly different for different computer stations.

The following instructions apply to both programs. Upon starting the program, follow the simple instructions supplied on the screen. Regardless of where you are in the program, the available options are always given. The <F1> key can be pressed at any time to get information that may be helpful (this is usually not necessary) in explaining the given assumptions of the simulation and how to proceed.

After getting beyond the introductory screen by pressing any key, the screen will display three boxes. The small one with <F> key information is self explanatory. The print screen command must be ignored if there is not a printer hooked up to your computer station.

The larger box on the left provides a set of choice commands. Any command can be implemented by pressing the key of the letter indicated in front of the command. Alternatively, use the up/down arrow keys to access the desired command and then press the <ENTER> key. The text at the bottom of the screen also describes how to implement these commands or quit the program. In all programs, the <e> command actually runs the experiment according to the PARAMETERS listed in the box on the right. By running the experiment, you will see the computer-produced electrophysiological data, just as it would appear on the oscilloscope screen of an actual electrophysiological rig.

The bottom four commands (<view screen>, <tune program>, <save parameters>, <load parameters>) are the same in each program. They are usually not necessary for the purposes of this lab exercise. However, if for any reason you want to save a particular set of experimental parameters so that they can be easily accessed later on, use the <save parameters> and <load parameters> commands.

All the other commands allow you to access one of the parameter categories seen in the large box on the right (i.e., ion concentrations, stimulus parameters, display parameters, etc.). Once implemented, you can change the value of the desired parameter within that category. You will either supply a <y> (yes) / <n> (no) input or a numerical value. It will be obvious which type of input is required. *You must press <ENTER> in order to implement any parameter change.* As indicated by the instructions at the bottom of the screen, the up/down arrow keys are used to access the different parameters within the category. To access another parameter category, one must first use the <ESC> key to return to the command list or primary menu.

Upon running the experiment (<e>), the electrophysiological data appears on the screen. At this point, there are instructions provided on the right hand side which provide one of three choices:

1. Make measurements of your data by using the cursor. Simply guide the cursor with the up/down/left/right keys along the waveforms, and the computer will read off the various time, voltage, current, and conductance values which relate to the individual traces. Note that the traces are color-coded, and the color key is located on the bottom right. If the cursor is advancing too gradually, change its *gain* by using the <+> and <-> keys.
2. Press <c> to clear the screen and return to the command / parameter screen.
3. Press the <ESC> key to return to the command / parameter screen. However, you must return to the last experiment screen using <v> and clear it with <c> before proceeding to the next experiment. This option is used when you want to go quickly back and forth between the parameter information and the electrophysiological data.

## Procedures

### CABLE.EXE

CABLE.EXE simulates the passive, non-spiking conduction properties of a neuron (Heitler, 1988) into which four microelectrodes have been placed along its axon length. One electrode serves as the stimulating electrode while the other three serve as the recording electrodes. The response measured is that of a graded response, one that varies with the intensity and time course of the stimulus and spreads without regenerating itself. CABLE.EXE allows you to alter the position of the recording electrodes, as well as the amplitude and duration of the current pulse. The program also allows you to change the axon parameters, (i.e., the membrane characteristics and/or diameter of the axon) then run the resulting simulation.

1. From the menu, press <2> and <ENTER> for entry into the **Infinite Cable Model**. Press any key.
2. This model will be set for experimental parameters for a passive or graded response of a non-spiking conduction property of a neuron. Note the experimental parameters under stimulus, display scales, and electrode position. Run the experiment by pressing <e>.
  - (a) Acquaint yourself with the position of the current stimulus, the amplitude of the response, and the resulting pulses recorded at each of the recording electrodes. There will be two pulses shown.
  - (b) For the purposes of the first part of this exercise, we will need to observe just one response. To accomplish this, press <c> to clear the experiment, and <ESC> to return to the experimental parameters. Press <u> to change the stimulus. Using the arrows move down to stimulus interval and *set it to 0.0 ms*. Press <ENTER>. Press <ESC>.
  - (c) Press <e> to run the experiment. You will now see just one response. Measure the voltage of the response, the current stimulus time base and the duration of the response by moving the cursor (now located in the upper right corner of your screen) using the arrows. You can read the results directly off the right hand side of the screen.
  - (d) Press <ESC> but do not clear the above pulse. Press <u> and change the pulse amplitude to 2.0 nA and press <ENTER>. Press <ESC> and then <e> to run the experiment. Note the superimposition of the two traces (one with pulse amplitude of 1.0 nA and one with 2.0 nA). Measure the differences in the amplitude between the various recordings. What are the effects of changing the pulse amplitude?
  - (e) Press <c> to clear the very first set of parameters, and then <ESC>. Now change the pulse width of the stimulus by pressing <u> and using the arrows to get to pulse width. Change the width to 10.0 ms, and press <ENTER>. Press <ESC> and then run the experiment by pressing <e>. What are the effects of changing the pulse width? Use the cursor to make your measurements.

- (f) Return to the default values (see Appendix B) and run the program with two stimuli. Is there any effect on the second pulse when the amplitude or duration of the stimulus are altered. Why?
3. You should now have a good command of the software, so detailed instructions for each step will not be given. Change the position of the electrodes by increasing the distance from the initial stimulus to the last electrode. Run the simulation. What does this tell you about a graded response? Would this response be indicative of an axon that can transmit stimuli over a long distance?
  4. Build your own experimental axon by altering the membrane characteristics and dimensions. Change the axoplasmic resistance, membrane resistance, membrane capacitance and axon radius. Run the simulation changing each parameter separately. Which of these parameters affects the response of the axon more? Why? (Your instructor will assist you in establishing plausible experimental parameters.)

### HH.EXE

This program simulates the active properties of a region of what is called a space-clamped axon according to the Hodgkin-Huxley model. The simulation can be run in two modes: unclamped and voltage clamped. In the unclamped mode, two current pulses of specified amplitude and duration are injected into the axon with a specified interval between them. The membrane voltage response, and the current and conductance changes underlying the response, are displayed. This enables phenomena such as the relationship between threshold stimulus strength and duration, and the refractory period, to be demonstrated, as well as revealing the ionic mechanism producing an action potential. In the voltage clamp mode the holding potential, clamp potential, and clamp duration can be specified. This enables the student to repeat the classic Hodgkin-Huxley experiments, and achieve a detailed understanding of the voltage-activated ion channels. In both modes, the effects of the drugs TTX, TEA, and veratridine can be simulated, to show the effects of blocking particular ion channels or gate mechanisms. The external concentrations of sodium and potassium ions can be altered (Heitler, 1988).

The simulation can also be run to show a “model” patch of a membrane containing a single sodium and potassium channel, whose gates open and close in a probabilistic manner. A graph of the activation and inactivation variables is shown, as is the sodium and potassium single channel conductances.

1. Upon entering the program, note that you are *not* recording in voltage clamp mode (i.e., you are in current clamp mode). Briefly scan the experimental parameters and then run the experiment as is. As with the previous experiment, we will do our first set of observations on a single pulse. We can achieve this by changing the stimulus amplitude of the second pulse to 0.0  $\mu\text{A}$ . (You should now know the steps to do this.)
  - (a) Observe the various parameters defining the action potential generated (graded response, threshold stimulus, depolarization, and repolarization).
  - (b) What are the ionic properties of the above parameters? Where do the voltage regulated sodium and potassium channels become activated? Why do they become activated?
  - (c) Press <c> to clear and then <ESC>. Run the model by pressing <m>. View the display and try to determine the various parameters illustrated.
  - (d) Return to the experimental parameters and try changing some of the parameters affecting the action potential, (i.e., the concentrations of the external ions or the strength of the stimulus). What are the effects of these changes? You can run the model after making any of these changes.
2. Return the parameters to the initial values displayed when you first entered HH.EXE (see Appendix B). Press <ENTER> and then run the experiment.

- (a) Measure the amplitude of the first and second evoked action potentials, using resting membrane potential as the baseline.
  - (b) Why might the second action potential have a lower amplitude than the first? What does this tell you about the inter stimulus interval? Is it within the absolute or relative refractory periods?
  - (c) Using a maximum stimulus pulse of 500  $\mu\text{A}$  for the second pulse, determine the *absolute refractory period*. *Hint*: Try different stimulus intervals!!
  - (d) Change the value of the second stimulus pulse to 50  $\mu\text{A}$  (the same as the first) and determine the *relative refractory period*.
3. Turn off the second stimulus pulse again and test the effects of the three different drugs separately. For each one, describe the effect on the action potential waveform and then determine the specific effect of each drug by running the membrane model command (<m>). *Hint*: Remember that voltage-gated sodium channels have separate activation gates (m gates) and inactivation gates (h gates), while voltage-gated potassium channels (delayed rectifier potassium channels) only have the activation gates (n gates).

In the presence of veratridine, why does the membrane potential remain at such a depolarized level?

4. Remove the drugs and test the effect of doubling the concentration of extracellular potassium (from 10 to 20 mM). Did the stimulus evoke multiple action potentials? Why?
5. Try the same thing using a 30 mM potassium concentration. How many action potentials were evoked? Why? *Hint*: Take a look at the membrane model (<m>).
6. Switch into voltage clamp mode, adjust the time display to 15 ms, change clamp duration to 20 ms, return the  $\text{K}^+$  concentration to 10 mM, and run the experiment. Measure the amplitude of the peak inward  $\text{Na}^+$  current, peak inward total current, and peak outward  $\text{K}^+$  current.

Why is the peak inward  $\text{Na}^+$  current different from the peak inward total current?

Why is the peak outward  $\text{K}^+$  current equal to the peak outward total current?

Measure how long it takes for the  $\text{Na}^+$  and  $\text{K}^+$  currents to reach their peak values.

7. Using a holding potential of  $-70$  mV, a clamp duration of 10 ms and a display timebase of 15 ms, construct a Current/Voltage relationship (I/V curve) for peak sodium current. *Hint*: Do repeated trials, each time incrementing the clamp potential by 10 mV. Begin at a holding potential of  $-50$  mV and go up to  $+60$  mV. Measure peak currents and peak conductances at each clamp potential. You will need the conductance measurements for the next step.
8. Plot the peak sodium conductance ( $g_{\text{Na}}$ ) and peak potassium conductance ( $g_{\text{K}}$ ) as functions of voltage.
9. Change the holding potential to  $-100$  mV and clamp potential to  $+40$  mV and run the experiment. Notice that there is a  $\text{K}^+$  current which occurs after the voltage clamp returns to the holding potential. Thus current is called a “tail current.”

Why does this current occur? Why is it in the “inward” direction? Why is there no  $\text{Na}^+$  tail current?

10. Change the holding potential to  $-40$  mV and re-run the experiment. What happened to the  $\text{K}^+$  equilibrium potential?

### Acknowledgements

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## APPENDIX A

### *Background Theory*

The following background material would be considered the minimal theory necessary for students to understand and operate *Neurosim*. A good reference for this theory is Eckert et al. (1988). This program is also suitable for advanced students in physiology with a far greater knowledge of neurophysiological functioning. An excellent reference for advanced students in neurophysiology is Kandel et al. (1991).

#### **Basic Properties and Components of Neurons**

The basic functional unit of any nervous system is the neuron. It is a cell specialized in the rapid conduction of information. It can maintain itself, receive information (which can be from other neurons), and transmit information to other neurons or target effector organs or cells.

A neuron consists of (1) a cell body, which is the nutritive region of the cell; (2) short branching processes called dendrites (which can be numerous); and (3) a long process called an axon, which is the main conductive part of the neuron for the transmission of nerve impulses. Nerve impulses are carried along the axon as waves of rapid change in electrical charge distribution across the membrane of the axon (called action potentials). Information is usually received directly by the cell body or via the dendrites.

Axons end in nerve terminals which are situated in close approximation with the target (another neuron or effector). The terminal is separated from the target by a very small gap called the synapse. Transmission is passed on to the target across the synapse. Nerves are bundles of axons bundled together in connective tissue.

Neurons are commonly classified according to function as (1) afferent (carrying information inward) or sensory, (2) efferent (carrying information away) or motor, and (3) interneurons (connecting neurons with other neurons).

In terms of general morphology, neurons can be classified as (1) unipolar (cell body off to one side of a long axon, e.g., some vertebrate sensory neuron), (2) multipolar (multiple dendrites converging onto the cell body with one axon leaving the cell body — useful in integrating incoming information from multiple sources), and (3) bipolar (singular process leading to the cell body and one single process moving away from it, e.g., invertebrate sensory neuron).

In primitive invertebrates, the neurons are mainly naked. Neurons of higher animals (particularly neurons in the central nervous system) are often associated with non-nervous cells called neuroglia that may play nutritive, supportive, or regulative functions.

In vertebrates and some higher invertebrates, axons of the peripheral nervous system are usually enveloped in a sheath formed by a particular type of non-nervous neuroglia, Schwann cells, around the neuron. Schwann cells contain myelin (a fatty or lipid substance) which has good insulating quality. By wrapping around the axon tightly a number of times, a Schwann cell forms a myelin sheath around the axon, insulating it electrically. Such neurons are called myelinated neurons.

The myelin sheath is discontinuous, and is interrupted at 1–2 mm intervals by nodes of Ranvier, where successive Schwann cells come together but leaving a minute gap that exposes the axon. The arrangement of the Schwann cells and interrupted myelin sheath is important for rapid nervous transmission as will be discussed later. In the central nervous system, the oligodendrocytes perform the same function as the Schwann cell in forming a myelin sheath.

#### **Neuronal Membrane Properties at Rest (Resting Membrane Potential)**

Nerve impulses are changes in the charge distribution across the membrane of the axon. Therefore, it is important to understand the membrane properties and electrophysiological properties of the neuron in order to understand the principle of how neurons function.

Like all living cells, there is an imbalance in the distribution of charged particles (ions) across the plasma membrane of neurons at rest. This imbalance in ionic distribution is physically measurable by microelectrodes as a charge difference or potential difference across the membrane. At rest, this potential difference is called the membrane resting potential.

The average resting membrane potential for vertebrate neurons is around  $-70$  mV. (The range is usually between  $-30$  to  $100$  mV for other cells depending on the cell type.) That means there is relatively more

positive charges outside of the membrane than inside the membrane. How does this come about? This is mostly due to the imbalance in the distribution of potassium and sodium ions and the presence of various types of membrane ion channels and active transport pumps for sodium and potassium. There is more  $K^+$  inside the cell cytoplasm than in the extracellular fluid outside the cell (about 35 times). Conversely, there is more  $Na^+$  (about 10 times) outside than inside. (The chloride ion concentration outside the cell is also higher outside than inside and this is in part due to the attraction to the positively-charged sodium ions.)

The plasma membrane of neurons at rest is selectively permeable to  $K^+$  (because of the presence of passive ion-specific channels in the membrane that are open at resting conditions) and relatively impermeable to  $Na^+$  and  $Cl^-$ . Potassium will tend to move from the inside to the outside down its concentration gradient. Since chloride ions inside (as well as the other anions and negatively charged proteins which are not lipid soluble) cannot follow to balance out the flow of positively-charged potassium ions, there is a build-up of a positive charge outside the membrane. Gradually, the accumulation of a positive charge outside the membrane will allow more potassium ions to passively diffuse outward through the channels as the electrical force balances out the potential force of the concentration gradient.

Although at rest the permeability of the membrane to sodium is low, there is still a very small leak for sodium in the membrane. A very small number of passive sodium channels are open and sodium ions tend to slowly “leak” or enter into the cell down its concentration gradient. The sodium ions that leak in are pumped back out via the sodium-potassium active transport pump. This pump uses ATP as an energy source and pumps three sodium ions out in exchange for two potassium ions coming in. This pumping in of potassium helps to keep the concentration of potassium ions high inside and also helps to create a net loss of positive charge to the outside, making the inside more negatively charged because of unequal exchange of sodium and potassium. This will, in part, contribute to the resting membrane potential.

In short, the major portion of the negative resting potential of the neuron (or any cell) arises directly from the high internal potassium ion concentration relative to the extracellular potassium ion concentration and to the tendency of potassium ions to leak out of the cell through many potassium channels, leaving behind a net negative charge. Although other ions like the sodium ion do not contribute directly to the resting potential since there are relatively few open passive sodium channels, it is an important, though indirect, participant. The active transport of the sodium ions to the outside in exchange for potassium ions via the sodium-potassium ATP pump, maintains a high intracellular potassium concentration, allowing potassium to be the predominant ion inside the cell. In addition, a small portion of the resting potential is a direct result of the pumping of positive charges ( $Na^+$ ) to the outside.

### **The All-or-None Action Potential (Nerve Impulse)**

Besides passive ion channels or leak channels, there are also voltage-gated channels specific for sodium and potassium, respectively, in the membrane. Voltage-gated channels are channels whose opening and closing are influenced by the potential difference across the membrane. Under normal resting conditions, the voltage-gated sodium channels and the voltage-gated potassium channels are closed.

When an axon is activated by a stimulus of sufficient strength from the receptive region of the neuron, the axon fires off a rapid, all-or-none action potential. At the level of the neuronal membrane, this stimulatory stimulus is usually translated in the form of a membrane depolarization, (i.e., membrane potential rising from its negative value towards zero or becoming more positive towards zero). The result is an action potential, a brief rapid change in membrane potential that travels along the plasma membrane of certain nerve and muscle cells.

Consider a small section of an axon. When the membrane of an axon becomes depolarized to a certain point, voltage-gated sodium channels begin to open. Sodium ions start to flow in, resulting in further depolarization which will cause all the voltage-gated sodium channels to open widely. This results in a rapid inflow of the positively-charged sodium ions leading to a rapid reversal of the membrane potential in that region of the axon, thus becoming relatively more positive inside.

Rapidly, the membrane becomes more depolarized as the sodium inflow voltage-gated potassium channels begin to slowly open. At the same time, the voltage-gated sodium channels begin to close again and sodium ions stop flowing in. The total duration of the transient sodium gate opening is 0.2 msec or less.

Due to the kinetics of their opening, the voltage-gated potassium channels only become fully open after the voltage-gated sodium channels have closed. With the potassium gates open, potassium ions rapidly exit

the cell carrying out positive charges. The membrane again becomes relatively more negative inside than the outside, thus restoring the resting membrane potential.

As the membrane becomes repolarized (gaining back the polarized state), the voltage-gated potassium channels begin to close once again. Since the potassium gates close relatively slowly, the membrane actually becomes temporarily more negative inside than at normal resting state; it transiently becomes hyperpolarized. This entire rapid change in membrane potential due to the rapid shifts in the normal sodium and potassium balance inside and outside the cell is the action potential. The entire duration is 0.5 msec or less.

Once the action potential is triggered, the changes in the membrane potential and the ion flows play themselves out automatically. The magnitude and duration depends only on the concentrations of the ions and the properties of the voltage-gated channels, these factors do not change in a normal healthy animal. As a result, action potentials are all the same in a given axon. They occur completely or not at all, thus *all-or-none*. In other words, there exists a threshold of action potential firing. As long as a stimulus depolarizes the membrane beyond the threshold, the all-or-none action potential will occur. The threshold of action potential firing ultimately depends on the properties of the voltage-gated sodium channel.

### Impulse Propagation and Neuron Recovery

How does an action potential travel down the axon? The intracellular compartment or cytoplasm is continuous, as is the interstitial fluid compartment immediately outside the axon. Depolarization of one part of the membrane and the resulting change in ion distribution will create a local flow of charges in the immediate neighbouring regions. This will tend to depolarize the membrane in this area. When the depolarization reaches the threshold in this neighbouring membrane, an action potential will also occur. Thus the action potential will propagate itself down an axon.

Why is it that a nerve impulse usually only travels in one direction and not reverses upon itself? Once a voltage-gated sodium channel opens and closes, it becomes inactivated, it cannot be opened again, until the membrane is repolarized to the resting membrane potential. Thus a local piece of neuronal membrane will be in a brief refractory state, and cannot be excited right afterwards. Thus, a nerve impulse cannot reverse upon itself. The period of time during which another action potential cannot be generated is called the refractory period.

Since a nerve impulse usually originates from the dendrites and cell body, the action potential can only travel down the axon in one direction. But, if the axon is stimulated artificially in the middle, an action potential will travel in each direction along the axon. Normally nerve impulses travel in one direction in a nervous system because transmissions across synapses (junctions) are usually one-way.

If a stimulus is strong and long lasting, it may remain above threshold strength for quite a duration. In this case, an axon will fire a second action potential after the refractory period, or a train of repeated action potentials, one after another. Since the action potentials are of the same size, signals transmitted along neurons can only be coded by frequency instead of amplitude.

### Factors Influencing the Speed of Conduction

Fast conduction is paramount especially if they coordinate important functions such as escape responses. Transmission of nerve impulses is basically in the form of the propagation of action potentials. The rate of propagation depends on the ease of local charge flow between neighbouring regions. Axons of larger diameter will have lower resistance to these local charge flows. Therefore, axons of larger diameter will have a faster rate of conduction of nerve impulse. This is used by a number of invertebrate species, (e.g., squid giant axon can be as wide as 1 mm, with a conduction velocity of 35 m/s). Some lower vertebrates use this approach too (e.g., some fishes and amphibians have unmyelinated axons of 50 mm vs. normal average of 10 mm).

Another way to increase the speed of conduction is achieved by the incorporation of highly insulative myelin sheaths and uninsulated nodes. A myelin sheath effectively stops ion movements across it. Action potentials are only generated at the node of Ranvier where the axon membrane is exposed to the interstitial fluid. The depolarization of one node causes, almost immediately, the depolarization of the next node. The ion flow is between nodes. Thus, the action potential essentially jumps from node to node. Conduction is

saltatory. By skipping sections of the axon, the speed of conduction is increased. Thus for any given diameter, myelination greatly improves the speed of conduction (a 10 mm myelinated axon can have a conduction velocity of 120 m/s).

### Transmission Across a Synapse

At the end of the axon, the terminal forms a synapse with the next cell, which can be another neuron, muscle cells, or endocrine cells. Synapses are the control switches of the nervous system. Synapses are not only junctions where information passes from one neuron (presynaptic neuron) to another cell (postsynaptic cell or postsynaptic neuron), it is also the place where the signal flow can be enhanced, retarded, or stopped.

There are two kinds of synapses: electrical and chemical. In electrical synapses, the two cells are joined by gap junctions. An action potential spreads directly across from one cell to another. They are adapted for rapid transmission of nerve impulse, but little modification of the signal is believed to occur with such a synapse.

Chemical synapses are more common. The terminals of the presynaptic neurons are unsheathed and are usually enlarged to form knobs. Each knob abuts on to a dendrite or cell body of another neuron. Between the presynaptic side and the postsynaptic side is a small gap, the synaptic cleft.

In the presynaptic side of the nerve terminal, there are many synaptic vesicles containing chemicals called neurotransmitters. When an impulse arrives at a nerve terminal, the transient depolarization causes the brief opening of voltage-gated calcium channels. Since extracellular free-calcium concentrations are higher outside than inside the nerve terminal (at least a difference of 10,000 times), calcium rushes inside the nerve terminal. This increases the free-calcium levels in the cytoplasm of the terminal for a short time. Increases in free-calcium levels causes the fusion of the synaptic vesicle to the presynaptic membrane and the release of neurotransmitters into the synaptic cleft. The calcium is removed from the presynaptic cytoplasm by several means and the free-calcium level is quickly restored without additional stimulation, thus stopping further transmitter release.

The neurotransmitter diffuses across the narrow synaptic cleft and binds to specific receptors on the postsynaptic membrane surface. Activation of the receptor on the postsynaptic membrane by the neurotransmitter opens chemical-gated ion channels, altering the membrane potential of the postsynaptic membrane. Two things can happen. If depolarization occurs (e.g., opening of sodium channels), we call such synapses excitatory because the membrane potential is brought towards the threshold and will fire off action potentials if it reaches threshold (e.g., acetylcholine synapses), and the changes in the postsynaptic potential, Excitatory Postsynaptic Potential (EPSP). If a hyperpolarization occurs (e.g., opening of potassium channels), we call such synapses inhibitory (the postsynaptic potential is brought further from the threshold, e.g., GABA synapses), and the changes in the postsynaptic potential, Inhibitory Postsynaptic Potential (IPSP). IPSPs and EPSPs are graded, depending on the size of the stimulation or the number of receptors activated.

Let us consider an example of an excitatory synapse and an excitatory neurotransmitter (e.g., acetylcholine). Acetylcholine diffuses across the synapse and binds to acetylcholine receptors. The acetylcholine receptors in the postsynaptic membrane of excitatory synapses are actually sodium ion channels. Binding of acetylcholine to its receptor opens the sodium channel in the middle of this receptor molecule. Inflow of sodium depolarizes the postsynaptic membrane. If enough acetylcholine receptors are activated, the resulting total sodium influx will generate a depolarization that will exceed the threshold for action potential firing and the postsynaptic neuron will generate an action potential.

Within the synaptic cleft is an enzyme (acetylcholine esterase) that breaks down acetylcholine to acetate and choline. So when acetylcholine dissociates from its receptor, it will be broken down and removed. Acetylcholine receptors are no longer stimulated and their sodium channels close. Potassium channels open to restore the postsynaptic potential to resting levels.

Thus, the degradation of acetylcholine by acetylcholine esterase helps in terminating the transmission. Some acetylcholine molecules released from the presynaptic vesicles may never reach the receptors because they can be degraded even before reaching the receptors. Acetate and choline are taken back into the presynaptic terminal for recycling. In some chemical synapses, the neurotransmitter is not broken down by enzymes but is taken up intact into the presynaptic terminal.

### Transmission Modification at the Postsynaptic Area

Temporal and spatial summation are two of the ways that transmission across the synapse can be enhanced, retarded, or otherwise modified at the postsynaptic membrane. Temporal summation is the phenomenon of the addition of the effects of two impulses that arrive within a short time interval. For example, the first impulse arriving at the presynaptic terminal may release insufficient neurotransmitters to bring the postsynaptic potential to the threshold for firing. However, if a second impulse arrives shortly afterwards, the additional neurotransmitter released by the second impulse may bring the concentration of the neurotransmitter in the synaptic cleft to a high level to occupy sufficient receptors to cause a large enough EPSP to be generated to cause the firing of an action potential. Alternatively, the first EPSP may not have decayed completely, and the second EPSP (although again smaller than that which is required to reach threshold alone) can add on to the first one. Together, they bring the depolarization to threshold level resulting in an action potential. So, the first impulse facilitates the second impulse in this example.

Spatial summation is the phenomenon of the additive effects of the first impulses affecting two closely spaced areas in the postsynaptic membrane. For example, two synapses are located close to one another. The PSP generated by one synapse will cause ion flow from its neighbouring membrane, thus affecting the membrane potential for a short distance around the postsynaptic area. If the second synaptic site is close enough to the first one, the effects of the first synaptic transmission on its postsynaptic membrane will affect the resting potential in the region of the second postsynaptic membrane such that if the second nerve terminal fires, its resulting PSP will be affected. For example, if the first synapse is inhibitory, it causes a hyperpolarization. This will also cause a slight hyperpolarization at the second postsynaptic site so that transmission across the second synaptic site will be impaired if the second terminal is firing at or slightly after the first one. Conversely, if the first synapse is excitatory, the membrane potential at the second postsynaptic site may be brought much closer to the threshold value so that a small EPSP at the second synapse can already fire an action potential. In this case, transmission at the second synapse is enhanced by the first synaptic site.

APPENDIX B  
Default Values

The following values are for the initial simulations (default values) in CABLE.EXE and in HH.EXE. Throughout the exercise you will be asked to change these values. You will also be asked to return to these values while carrying out the exercise.

**CABLE.EXE**

Stimulus		Display Scales	
pulse amplitude	1.0 nA	voltage	3 mV
pulse width	5.0 ms	current	2.0 nA
interval	20.0 ms*	timebase	30.0 ms
Electrode position		Display (y = yes, n = no)	
E1	1	y	
E2	2	y	
E3	4	y	
*will give two peaks; change to 0.0 ms for one peak.			

**HH.EXE**

Stimulus (u) (Voltage clamp OFF)				
	1st	2 <sup>nd</sup>		
amplitude	50.0	250.0 $\mu$ A		
width	0.25	0.15 mS		
interval	7.00 m/s			
Drugs applied (G)		External ion conc. (i)		
TTX	N	Na	418 mM	
TEA	N	K	10 mM	
veratridine	N			
Display features		Y=on N=off		
	Na	K	Total	Scale
conductance	y	y	-	50
ionic current	y	y	y	+/-1000
stimulus current				500
voltage display range	40 to -80			
timebase	10 ms			

APPENDIX C  
*Ranges of Acceptable Values from Heitler (1988)*

**CABLE.EXE**

**Building an Axon**

When building your axon, the following acceptable ranges should be kept in mind:

Axoplasmic resistance ( $R_i$ ): 0.02 – 0.5 kOhm  $\text{cm}^2$

Membrane resistance ( $R_m$ ): 0.1 – 50 kOhm  $\text{cm}^2$

Membrane capacitance ( $C_m$ ): 0.1 – 10  $\mu\text{F}/\text{cm}^2$

Axon radius: 0.11 – 500  $\mu\text{m}$

**Set Stimulus**

Two current pulses are automatically injected into the axon as stimuli. More pulses can be injected manually by pressing the space bar. The following are acceptable ranges:

Amplitude: 0 – 999 nA

Pulse width (this sets the duration of the injected pulses): 0 – 999 ms.

Pulse interval: 0.5 – 999 ms. Two stimulus pulses are triggered automatically. The first occurs at the beginning of the sweep, the second at the interval set by this parameter. Stimuli can also be triggered manually by pressing the space bar during the course of a run.

**Display Scales**

The following are acceptable ranges:

Voltage: 1 – 999 mV. Deflection is from the left.

Current: 1 – 999 nA.

Timebase: 1 – 999 ms. This sets the horizontal timebase. This is the duration of the screen display. If the timebase is set to less than about one tenth of the time constant, the display will become very coarse.

**HH.EXE**

**Stimulus**

This sets the stimulus parameters for a non-clamped axon, and the holding and clamp potentials for a clamped axon.

*Voltage Clamp OFF.* The unclamped axon can be stimulated with two current pulses. The following parameters can be specified:

Amplitude (sets the amplitude of the current pulses): 0 – 999  $\mu\text{A}/\text{cm}^2$ .

Width (this sets the duration of the current pulses): 0.04 – 99 ms.

## 170 Computer Simulation

Interval (this sets the interval between the current pulses): 0.04 – 99 ms. The minimum time is whatever the integration time step has been set at (0.04 ms, unless altered) and the program will adjust times to the nearest whole multiple of this value. To prevent the second pulse occurring, the amplitude is set to 0.

*Voltage Clamp ON.* This sets the holding potential, clamp potential and clamp duration for the voltage clamp.

Holding potential: –200 to +200 mV. The program assumes that the membrane has been held at this potential for a long time period prior to the experiment.

Clamp potential: –200 to +200 mV. The membrane is clamped at this potential at the start of the experiment.

Clamp duration: (this sets the duration for which the axon is maintained at the clamped potential): 1 – 999 ms.

### Ion Concentrations

This simulates changes in the external concentrations of sodium and potassium ions. The effect is as though the ion concentrations had been changed from their default values of 418 mM (sodium) and 10 mM (potassium) at the instant of the start of the simulation. The following parameters can be specified:

Na (sets the external concentration of sodium ions): 0 – 2000 mM.

K (sets the external concentration of potassium ions): 0 – 2000 mM.

### Display Scales

The following parameters can be changed:

Conductance (sets the scale for conductance display): 1 – 999 mSs.

Ionic current (sets the scale for the current display): 1 – 9999  $\mu$ A.

Stimulus current (this is only applicable in non-clamped experiments): 1 – 999  $\mu$ A.

Voltage display range:

The first parameter sets the voltage at the top of the scale): 0 – 22 mV.

The second parameter sets the voltage at the bottom of the scale: 0 to –200 mV.

Timebase (sets the duration of the display): 1 – 999 ms.