Chapter 4

Electrophysiology

After reading this chapter, you should be able to:

- Understand the fundamental electrical properties of neurons and the ionic basis of membrane potentials
- Describe the basic components of an electrophysiology rig
- Compare different categories of electrophysiological recording techniques, including which research questions each technique can best address
- Compare different types of tissue preparations used in electrophysiology experiments

Techniques covered:

- **Common electrophysiological approaches**: extracellular recordings, intracellular recordings, patch clamp techniques
- **Common electrophysiological preparations**: heterologous expression systems, primary cultures, brain slice cultures, anesthetized or awake animals

Electrophysiology refers to the study of the electrical properties of cells, including membrane potentials, action potentials, and the specific ion channels and other proteins that endow cells with their electrical characteristics. In the field of neuroscience, electrophysiology techniques can answer systems level questions, such as the role of a neuron in a circuit or behavior. Alternatively, these techniques can answer questions about the molecular and cellular properties that allow different neurons to exhibit unique physiological properties. Compared with other techniques used to measure neural activity using histological or optical methods (see Chapter 7), electrophysiology techniques directly measure electrical phenomena. Because electrical signaling is the fundamental basis of neural signaling and communication, electrophysiology techniques are highly valuable for understanding the nervous system.

Electrophysiology techniques are often categorized by where the recording instrument, the electrode, is placed in the neural specimen. In an **extracellular recording** experiment, an electrode is placed just outside a neuron of interest. In an **intracellular recording** experiment, an electrode is inserted inside a neuron of interest. Finally, using **patch clamp techniques**, an electrode is closely opposed to a neuronal membrane, forming a tight seal with a patch of

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the membrane. These different recording techniques are used to examine the electrical properties of neurons both in vitro and in vivo. In vitro recordings from cell cultures and brain slices allow for detailed investigations of the molecular bases of a neuron's physiological properties. In vivo preparations allow scientists to correlate a neuron's firing properties with animal behavior or other physiological phenotypes.

The purpose of this chapter is to differentiate between the major categories of electrophysiological techniques and preparations, comparing the relative advantages, disadvantages, and common uses of each. We will start with a brief review of the physiological principles that endow neurons with their electrical characteristics. To explain how electrophysiology techniques are performed, we will survey the instruments that make up an electrophysiology setup ("the rig"). We will also examine some common methods of data analysis and presentation used in the literature.

A BRIEF REVIEW OF THE ELECTRICAL PROPERTIES OF NEURONS

The electrical activity of a neuron is based on the relative concentration gradients and electrostatic gradients of ions within the cell and in the extracellular fluid, as well as the types of ion channels present within the neuron. The difference in charge between the intracellular and extracellular sides of the membrane creates an **electrical potential**, measured in units of volts (V). A neural membrane at **resting potential** is about −70 mV (meaning that the intracellular side of the membrane is −70 mV with respect to the extracellular environment). This resting potential is caused by differences in the relative concentration and permeability of various inorganic ions, particularly sodium (Na^+), potassium (K^+), and chloride (Cl^-), as well as by the active contributions of a sodium−potassium pump (Fig. 4.1). Ions moving across the membrane generate a measurable **current** (I), the movement of charge over time. The movement of ions across the membrane is limited by the membrane **resistance** (R). This resistance is generated by properties of the membrane, such as how many channels are open or closed. The relationship among the membrane potential, the current flow, and the membrane resistance is described by **Ohm's law**: V = I x R. This relationship is the fundamental basis of many electrophysiological techniques and methods of data analysis.

Neurons communicate by causing changes in membrane potential in other neurons. For example, most neurons communicate by releasing chemical neurotransmitters into a synapse, the physical space between two neurons. A presynaptic neuron releases neurotransmitters that bind to receptors on the postsynaptic neuron. The postsynaptic neuron expresses neurotransmitter receptors that cause the opening or closing of ion channels on the postsynaptic neural membrane, causing a change in membrane potential. Relative to the postsynaptic neuron's resting membrane potential, this current flow can cause

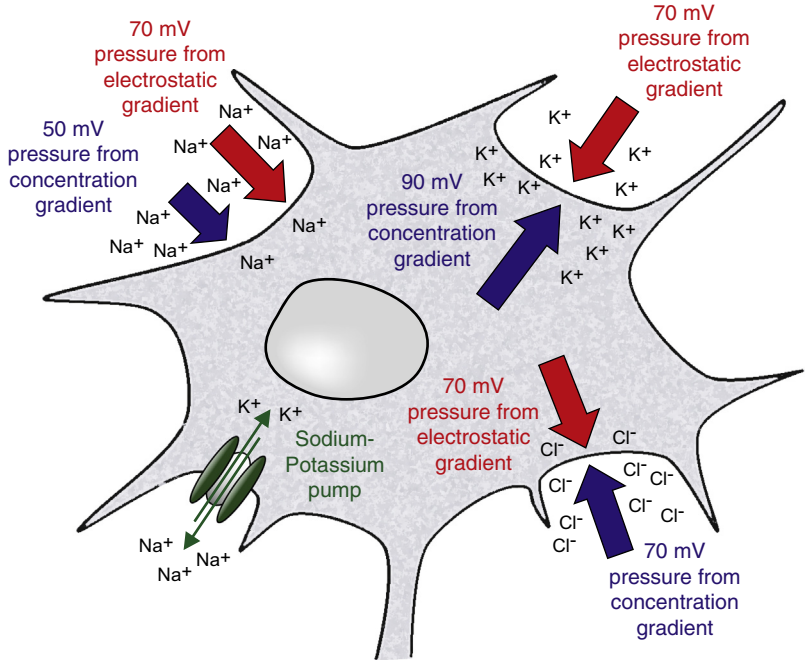


FIGURE 4.1 **The ionic basis of the resting potential.** The resting potential of neurons, about −70 mV, is caused by the relative concentration and permeability of various inorganic ions. These ions experience pressure to move in or out of the cell based on concentration gradients (differences in concentration of the ion per unit distance in the local environment) and electrostatic gradients (differences in electrical charge per distance in the local environment). In addition to passive diffusion, a sodium-potassium pump continually pumps sodium ions out of the cell and potassium ions into the cell.

the membrane potential to become more positive, an effect called **depolarization**. Alternatively, current flow can make the membrane potential more negative, an effect called **hyperpolarization**. These local voltage changes are called **graded potentials** or **localized potentials**, and their magnitude is proportional to the strength of the stimulus. A local voltage change that makes the membrane potential more positive is called an **excitatory postsynaptic potential (EPSP)**, while a local voltage change that makes the membrane potential more negative is called an **inhibitory postsynaptic potential (IPSP)**. Different EPSP and IPSP events combine to form an overall signal in the postsynaptic neuron. These localized potentials can add up in space (called "spatial summation") and time (called "temporal summation"). If enough localized potentials sum to depolarize the membrane to a threshold point, usually around −55 mV (called the "threshold potential"), an action potential will occur.

An **action potential**, also referred to as a **spike**, is an all-or-none, rapid, transient depolarization of the neuron's membrane. An action potential occurs when a local depolarization to the threshold potential triggers the opening of voltage-gated sodium channels, and the rapid influx of sodium ions brings the membrane potential to a positive value (Fig. 4.2). The membrane potential is restored to its normal resting value by the delayed opening of voltage-gated potassium channels and by the closing of the sodium channels. A refractory period follows an action potential, corresponding to the period when the voltage-gated sodium channels are inactivated. The all-or-none generation of an action potential initiates a wave of depolarization that propagates down the axon's membrane.

In a chemical synapse, depolarization causes the fusion of synaptic vesicles within the presynaptic membrane and the release of neurotransmitter molecules into the synaptic cleft. The neurotransmitters bind to receptor proteins associated with particular ion channels on the postsynaptic membrane. These ion channels then generate EPSP and IPSP events in the postsynaptic neuron, which can, in turn, sum to generate an action potential in that neuron.

Many more details on the electrical properties of neurons can be found elsewhere. What is important to appreciate in the context of understanding

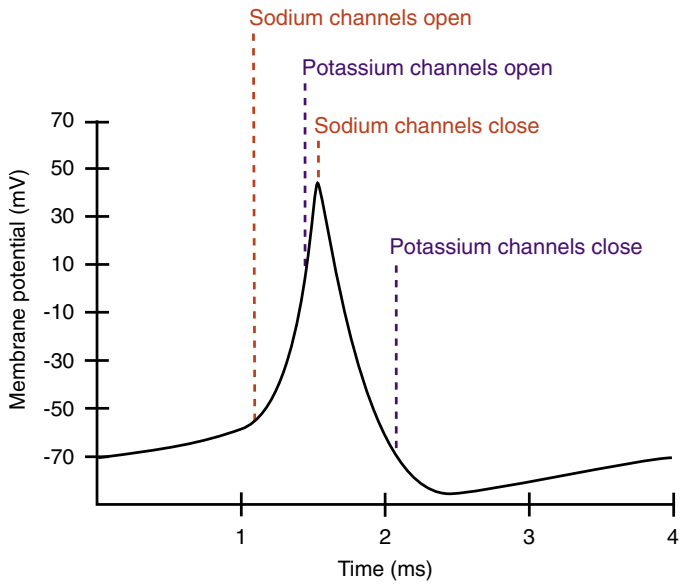


FIGURE 4.2 **The ionic basis of an action potential.** Localized potentials within the neuron sum to bring the membrane voltage to its threshold potential, around −55 mV. This potential causes voltage-gated sodium channels to open, further depolarizing the membrane. Potassium channels open as the membrane potential becomes more positive. At about 25 mV, sodium channels close, and the membrane potential decreases until it becomes hyperpolarized. Finally, potassium channels close, and the membrane potential returns to a resting state.

electrophysiology techniques is that scientists can study these properties at different levels of investigation. For example, an investigator may want to know the frequency of action potentials in a specific neuron over time to elucidate how a neuron encodes a particular stimulus or action. Alternatively, an investigator may want to know how the presence of a drug in the extracellular fluid affects the ability of a specific ion channel to pass current. Whether in the context of circuit analysis or the molecular basis of the membrane potential, nearly any aspect of neuronal physiology can be investigated with the electrophysiology methods described in this chapter. Before we describe these methods, let's examine the tools and equipment necessary to perform an electrophysiology experiment.

THE ELECTROPHYSIOLOGY RIG

Each electrophysiology lab setup is different, depending on the questions being addressed, the requirements of the experiment, and the personal preferences of the investigators. There is, however, a standard set of equipment necessary to record electrical signals from neurons (Fig. 4.3). In general, a

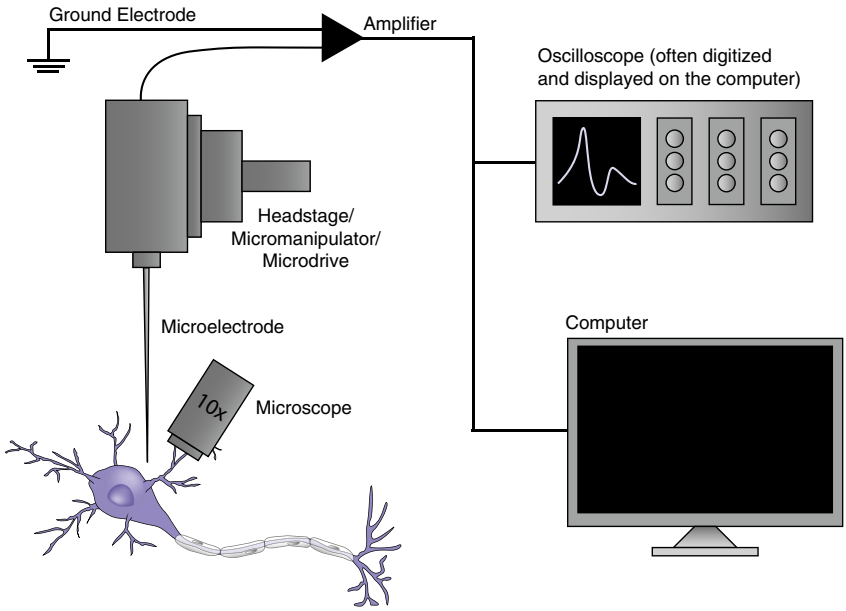


FIGURE 4.3 Some of the fundamental components of an electrophysiology rig. In general, an electrical signal is recorded by a microelectrode and passed along to an amplifier. The amplifier compares the recording to a ground electrode, then transmits the signal to an oscilloscope and/or computer. Various other equipment is necessary and desirable depending on the nature of the experiment.

signal is detected by a **microelectrode**, which transmits that signal to an **amplifier**, an **oscilloscope**, and a **computer**. The oscilloscope presents a visual display of the membrane potential over time, which can also be heard using a **loudspeaker system**. In modern electrophysiology rigs, the oscilloscope is now completely digital: an analog-to-digital converter transforms the raw electrical signal into a digital value that is displayed on the computer screen. An electrophysiology setup also includes equipment to stabilize the microelectrode and correctly position it to record from a neuron of interest. A **microscope** is used to ensure proper placement of the electrode for in vitro recordings, while stereotaxic equipment (Chapter 3) is used to position electrodes for in vivo recordings. Box 4.1 details various components of a standard electrophysiology rig.

BOX 4.1 The Electrophysiology Rig

The following items may or may not be part of a neuroscientist's electrophysiology rig. Each electrophysiology rig is set-up differently, depending on the needs of the investigator.

Microelectrode

There are two main types of electrodes: (1) glass micropipettes filled with an electrolyte solution (usually sodium chloride or potassium chloride) and (2) metal electrodes (usually tungsten, steel, or platinum–iridium). An important characteristic of both kinds of electrodes is their electrical resistance, which is related to the exposed tip size. Smaller tips have higher resistances, and they restrict the area from which potentials can be recorded, thus permitting the isolation of the activity of either a fiber or a cell. Large tips and low resistances pick up the activity from a number of neurons and are of limited use in efforts to identify the functional properties of single cells. Tips with very high resistances are also of little use, as they cannot record neural activity unless they are very close to the cell membrane or actually inside a cell.

Glass micropipettes are necessary for patch clamp recordings because of the way the pipette must make a tight seal with the cell membrane. For intracellular or extracellular electrophysiology experiments, metal electrodes are more commonly used. These metal electrodes not only provide more stable isolation of single units than micropipettes, they also tend to sample from a larger morphological variety of cells and also help in better localization of electrode tracks to identify where recordings took place in whole brains. The main advantage of using a glass electrode in extracellular or intracellular experiments is that the pipette can be filled with a dye or other materials that can be injected into the cell or local environment for visualization or pharmacological experiments.

A pipette puller is used to create glass electrodes. A glass capillary is loaded into the machine, which heats the glass and pulls it apart very quickly, creating two glass electrodes with very fine tips. For glass micropipettes that will be used in patch clamp recordings, the micropipette is also polished to round and smooth the electrode tip. Glass capillaries and pipette pullers come in many varieties and are commercially available. Metal electrodes are generally purchased ready-for-use.

BOX 4.1 The Electrophysiology Rig—cont'd

Note that two electrodes are necessary for any electrophysiological recording: the recording electrode itself and a reference electrode (also called a "ground" electrode) placed outside the cell of interest. A reference electrode is necessary because an electrophysiological measurement is a comparison—for example, a comparison of the potential difference across the membrane of a neuron. In extracellular electrophysiology, both electrodes are located outside the neuron but are placed in different locations in the extracellular environment.

Headstage

The headstage is the central hub that connects the electronic equipment to the tissue preparation. It contains an electrode holder that stabilizes the microelectrode during recordings and also directly connects the microelectrode to the main amplifier for initial signal processing. The headstage is carefully positioned by the micromanipulator and is also attached to the microdrive.

Micromanipulator

The micromanipulator is a device that allows fine movements in the X, Y, and Z axes, permitting precise positioning of the microelectrode in tissue. Good micromanipulators have fine-scale units of measurement (usually μm) and can be used to stereotaxically place the microelectrode in specific regions of brain or tissue.

Microdrive

A microdrive is used to lower or raise the microelectrode to a specific depth in tissue in very fine steps. It is usually preferable to use remote-controlled microdrive systems to eliminate hand vibration. Thus, the headstage (and consequently the electrode) can be set into place by hand using the micromanipulator and then finely adjusted in and out of tissue using a microdrive for the final approach to the cell.

Amplifier

The signal is passed from the microelectrode on the headstage to the main amplifier, where amplification of the signal takes place (usually 100—1000 times the original signal). An amplifier is necessary to enhance the relatively weak electrical signal (typically picoamps or nanoamps) recorded from neurons. The amplifier also receives signal from the reference electrode, and it is here that the signals from the two electrodes are compared. The amplifier then transmits the signal to an oscilloscope and/or computer.

Oscilloscope

An oscilloscope receives the electrical signal from the amplifier and displays the membrane voltage over time. In modern electrophysiology rigs, the raw electrical signal is digitized, and the oscilloscope is part of electrophysiology recording software on a computer. However, analog oscilloscopes may still be useful for detecting subtle electrical signals that can get accidently filtered out by a computer.

Loudspeaker System

Changes in voltage over time are visualized using an oscilloscope or digital oscilloscope, but they can also be heard by connecting the output of the amplifier

Continued

BOX 4.1 The Electrophysiology Rig—cont'd

to a loudspeaker so that action potentials make a distinctive popping sound (similar to the sound of popcorn popping). Loudspeakers can be helpful when trying to locate a neuron of interest, because different types of neurons have distinctive firing patterns. An investigator lowering an electrode into neural tissue using a microdrive can be alerted to a specific type of cell or group of nuclei by the characteristic sounds of neurons near the electrode tip.

Microscope

A microscope is almost always necessary for all kinds of physiological recordings. For extracellular recordings, a low-power dissecting microscope is usually adequate to see laminae or gross morphological features of the tissue and brain. For intracellular physiology or patch-clamp techniques, a microscope with high enough magnification power to see individual cells (300—400×) is necessary. These microscopes are usually equipped with optical contrast enhancement to view cells in unstained preparations. An inverted microscope is usually preferable for two reasons: (1) it allows easier electrode access to the sample, as the objective lenses are below the chamber and (2) it provides a larger, more solid platform upon which to bolt the micromanipulator.

Computer

Computers have greatly aided electrophysiological studies by automating stimulus delivery and electrical signal recording. Computer software makes it easy to write programs that can reproducibly introduce sensory or electrical stimuli to animals or tissue preparations and record neural responses. Computers can easily manipulate many parameters during recordings, such as the recording thresholds and stimulus delivery timing. Computers also allow simple real-time data analysis, as they can display the results of an experiment in real time.

Vibration Isolation System

A vibration isolation system, usually an air compression table, is used to absorb tiny changes in vibration that can disturb the placement of the microelectrode.

Faraday Cage

A Faraday cage is a simple enclosure made from conductive material that blocks electrical noise from outside sources (such as the electrical systems that power the lights in the room).

Other equipment may be necessary, depending on the exact nature of the experiment. For example, in experiments that study auditory physiology, a sound booth is necessary to block environmental sounds that could influence recordings. Pharmacological injection equipment is often included in a rig to administer drugs or other substances into neural tissue. Additional stimulus delivery systems may be necessary, depending on the stimuli needed for the recordings. Each scientist's rig is often a highly specific setup, with all of the equipment designed and calibrated for the particular needs of the experiments being performed.

CATEGORIES OF ELECTROPHYSIOLOGY RECORDINGS

Electrophysiological recordings can be grouped into three main categories based on the placement of the electrode in relation to the cell: (1) extracellular recordings; (2) intracellular recordings; and (3) patch clamp techniques (Fig. 4.4). Each technique can be used to address specific questions concerning the electrical properties of neurons. For example, questions about the firing properties of neurons in vivo are most easily addressed using extracellular methods. Questions regarding the "open" and "closed" states of a specific ion channel are best addressed using patch clamp techniques. Table 4.1 compares some of the questions that can be addressed using various types of electrophysiology recordings.

Electrophysiologists using intracellular or patch clamp methods have the benefit of being able to make use of closed electrical systems to illuminate the relationship between membrane potential and current flow: voltage clamp and current clamp. Using a **voltage clamp**, a scientist can measure currents generated by ions moving across the membrane by holding the membrane potential at a set voltage, the **holding potential**. Deviations between the membrane potential and the holding potential are corrected through a feedback system that injects current to maintain the membrane voltage at the holding potential. In this way, membrane potential is "clamped" to a set voltage. Data collected using a voltage clamp can be plotted in I/V curves (Box 4.2) to determine the current flow through a channel at a particular voltage over time (Box 4.3). In **current clamp** mode, membrane potential is free to vary, and the investigator records whatever voltage the cell generates on its own or as a result of stimulation.

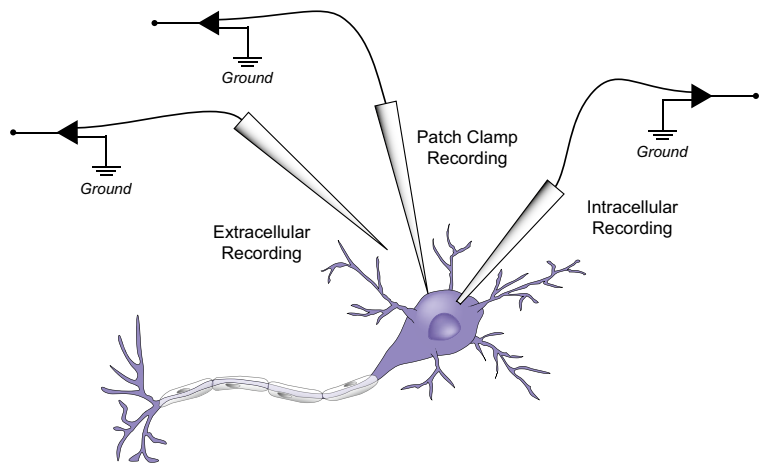


FIGURE 4.4 Three categories of electrophysiological recordings. Each type of recording is defined by where the scientist places the recording electrode: outside the neuron (extracellular recording), inside the neuron (intracellular recording), or adjacent to the membrane (patch clamp recording). Triangles denote the comparison of signals between the recording electrodes and ground (reference) electrodes.

TABLE 4.1 Questions That Can be Addressed Using Different Electrophysiological Techniques.

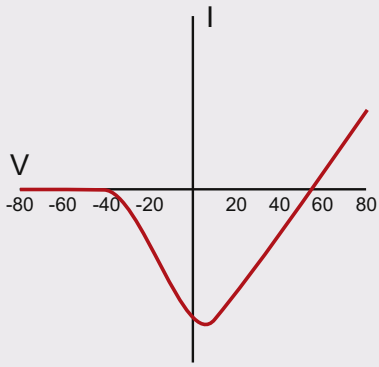
Extracellular recordings *Recording from the extracellular environment near a cell (or cells) of interest*	• How does a neuron encode information in action potentials? • How does the activity of a neuron correlate with behavior or other physiological parameters? • How does the activity (or inactivity) of one neuron affect the activity of another neuron? • How do pharmacological agents, neurotransmitters, and neuromodulators affect the firing of a neuron? • How is the spiking activity of a group of neurons coordinated?
Intracellular recordings *Recording from inside a cell of interest by piercing the membrane with an electrode*	• How does the activity (or inactivity) of one neuron affect the local potentials and action potentials of another neuron? • How do pharmacological agents, neurotransmitters, and neuromodulators affect the local potentials and action potentials of a neuron?
Patch clamp recordings *Recording from the membrane of a cell of interest*	• How do an ion channel's open and closed times depend on the membrane potential? • How do the concentration of ions, pharmacological agents, neurotransmitters, and neuromodulators affect the current flowing into an ion channel or cell? • How much current does a single ion channel carry? • What contributions does a single channel provide to an entire neuron?

BOX 4.2 Data Analysis: I/V Curves

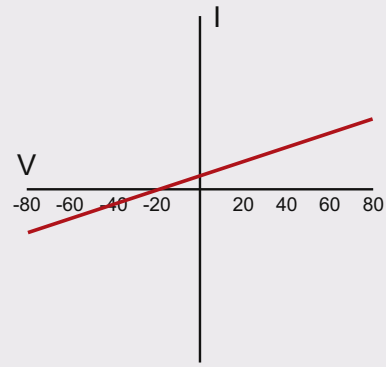
An **I/V curve** (current/voltage curve) is one of the most commonly used methods of data analysis in electrophysiology. It is simply a plot of the voltage across a neuronal membrane and the associated ionic current flow through a channel or channels in the membrane. Voltage (V) is measured in units of volts, and current (I) is measured in units of amperes. I/V curves can be produced for an entire neuron (with current typically in the range of nanoamps, nA) or for an individual ion channel within the neuron (with current typically in the range of picoamps, pA).

BOX 4.2 Data Analysis: I/V Curves—cont'd

I/V curve of a voltage dependent channel



I/V curve of a non-voltage dependent channel



To produce an I/V curve, an investigator uses the voltage clamp technique to hold the voltage of a neuron at a specific value. Current can be recorded using one of the patch clamp methods. The I/V curve is the curve of best-fit for each data point of current for a given voltage. By convention, currents with a negative value are referred to as "inward current," while those with a positive value are known as "outward current." An inward current is the result of positively charged ions crossing a cell membrane from the outside to the inside, or a negatively charged ion crossing from inside to outside. An outward current is the result of positively charged ions crossing a cell membrane from the inside to the outside, or a negatively charged ion crossing from the outside to the inside.

The relationship between current and voltage is described by Ohm's law:

$$V = I \times R$$

V = voltage
I = current
R = resistance
1/R = conductance

The slope of the I/V curve is the conductance of the membrane/channel for all ions that pass through. In studies in which the investigator is interested in the conductance of a specific ion, pharmacological agents are used to isolate specific channels. The conductance depends on an ion channel's permeability for a specific ion (how easily the channel allows the ion to pass through), as well as the concentration of the ion in the extracellular/intracellular solution.

In the examples above, the I/V curves could represent the current/voltage relationships for particular channels within a membrane. Note that the I/V curve for the channel on the right is linear, and the conductance of the channel is independent of voltage. An electrophysiologist would say there is no voltage-dependent gating of this ion channel. By contrast, the I/V curve on the left is not linear. At a value of about −20 mV, there is a strong inward current. If the ion

Continued

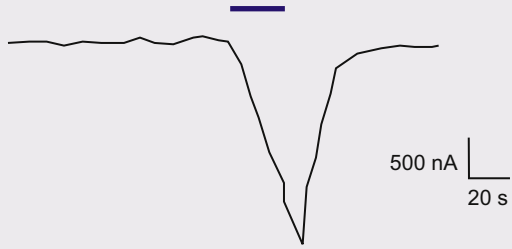
BOX 4.2 Data Analysis: I/V Curves—cont'd

that passes through this channel is positively charged, it will flow into the cell when the membrane potential is −40 to +60 mV. This is an indication that the channel is voltage gated. To determine the type of ion that can flow through this channel, the ionic concentrations in the bath solution can be adjusted so that the investigator can determine which ionic species are required for current to flow through the channel.

BOX 4.3 Data Analysis: Current Over Time

Many questions in neurophysiology involve asking how much current flows through a particular ion channel for a particular voltage or other environmental conditions. An I/V curve displays the relationship between the current that passes through a channel or membrane and the voltage across the membrane. For questions involving the ability of a cell or channel to pass current under conditions other than changes in voltage (such as the presence of a compound in the bath or the temperature of the bath), data are often presented as a plot of current over time. These measurements are typically taken under voltage clamp to eliminate contributions to current dynamics due to changes in voltage.

The amplitude of the current depends on the patch clamp technique used to collect the data. For example, in whole-cell mode, the glass pipette is continuous with the cytoplasm, and a large current trace can be obtained. Current data in whole-cell mode is typically presented in nanoamps and can be applied to timescales ranging from 1 ms to even minutes in length. In the following whole-cell recording, an agonist for a ligand-gated ion channel is applied to the bath, as indicated by the blue bar.

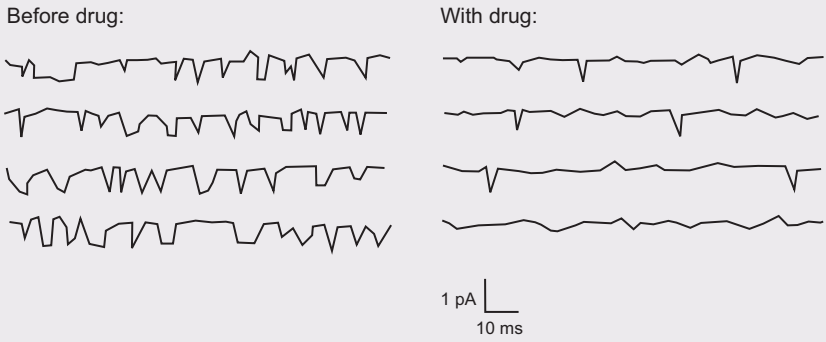


Current data obtained from other patch clamp modes are typically smaller in value and presented in picoamps because the current flowing through single channels rather than an entire cell generates a smaller signal. The timescale of

BOX 4.3 Data Analysis: Current Over Time—cont'd

these current traces is also much smaller, in the range of milliseconds instead of seconds, due to the rapid and transient durations of channel opening and closing.

For these patch clamp modes, data are often presented as a long, continuous trace. For example, in the following experiment, data are obtained in a cell-attached mode from a single channel before and after application of a pharmacological agent:



Notice how the scale is different from the whole-cell recording. This experiment shows that the presence of the pharmacological agent reduces the probability that the channel will be open and conduct current.

Extracellular Recording

The changes in membrane voltage that occur during an action potential generate local, temporary differences in potential on the outer surface of an active neuron. Thus, action potentials can be detected in the extracellular space near the membrane of an active neuron by measuring the potential difference between the tip of a recording electrode and a ground electrode placed in a distant extracellular position. In the absence of neural activity, there is no difference in potential between the extracellular recording electrode and the ground electrode. However, when an action potential occurs near the tip of the electrode, positive charges flow away from the recording electrode into the neuron. Then, as the membrane repolarizes, positive charges flow out across the membrane toward the recording electrode (Fig. 4.5). These extracellular measurements can be analyzed and visualized in a variety of ways (Box 4.4).

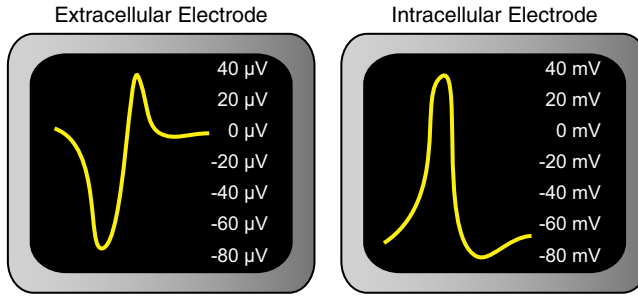


FIGURE 4.5 Comparison of recordings from an extracellular and intracellular electrode. Each action potential exhibits a different waveform due to its placement relative to a neuron. Note the differences in the units of measurement.

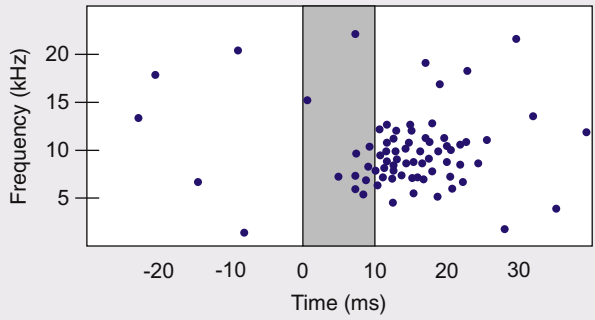
BOX 4.4 Data Analysis: Spikes

The goal of many electrophysiology experiments is to quantify the frequency of action potentials, or spikes, that a neuron fires in response to a specific stimulus. Usually, these experiments use extracellular recording electrodes, though intracellular electrodes and patch clamp techniques can also detect action potentials. Figures displaying changes in the number of spikes that occur over time following the presentation of a stimulus are presented in several common ways.

For example, consider the data collected during an extracellular electrophysiology experiment in which an electrode is placed in the auditory cortex of a mouse. The investigator records from a neuron close to the tip of the electrode to determine whether this neuron responds to tones of a particular frequency. The simplest way to present data from this experiment is to show a plot of voltage over time:

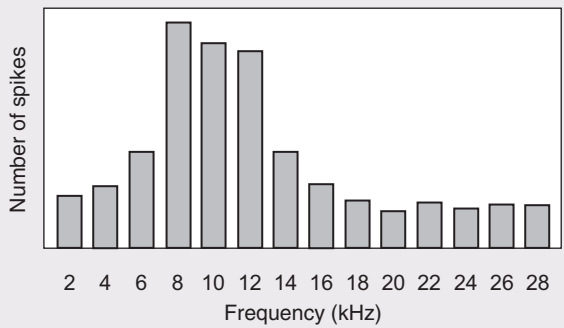
This plot clearly shows that the stimulus leads to specific changes in voltage over time. However, a simple voltage over time plot does not present the effects of multiple types of stimuli—in this case, multiple frequencies of sound. To show the effects of a continuous distribution of stimuli on spike counts, there are other, more efficient methods of data visualization that can combine the results of multiple experiments with different stimuli. For example, the results of multiple frequencies can be presented as a **raster plot**.

BOX 4.4 Data Analysis: Spikes—cont'd



In this type of plot, each dot represents a spike as recorded by the electrode. The *Y*-axis shows the stimulus frequency, while the *X*-axis shows the time when the spike was recorded. Stimulus onset and duration are indicated by the vertical gray bar. The data are arranged so that the spikes resulting from a stimulus are aligned with the onset of the stimulus. A raster plot provides a strong visual depiction of the kinds of stimuli that give rise to the greatest number of spikes, as well as an indication of the background noise during an experiment.

Data in a raster plot can also be presented as a **peri-stimulus time histogram** (PSTH) (also referred to as a poststimulus time histogram). In this type of plot, spikes are quantified and sorted into bins of a defined size. In the following plot, the frequency (X-axis) is presented for bins that are each 2 kHz in size, with the number of spikes plotted on the Y-axis. A PSTH provides an easy visualization of the stimuli that cause the greatest proportion of firing events. Different mathematical analysis tools, such as a Gaussian distribution curve, can be used to characterize the most effective stimuli and determine the tuning properties of an individual neuron.



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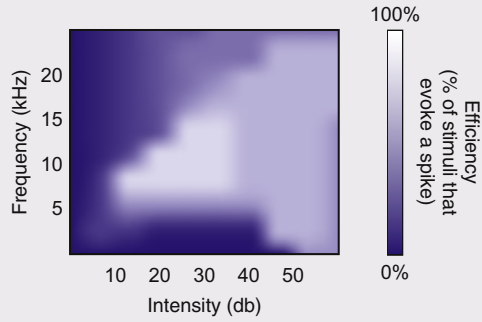
BOX 4.4 Data Analysis: Spikes—cont'd

Finally, it is possible to analyze the effects of multiple stimuli using a three-dimensional "heat map" plot. For example, if the investigator presented tones of different intensity in addition to tones of different frequency, the data could be presented using color to indicate the number of spikes.



This plot shows that the neuron being investigated responds to a specific frequency of tones that produce a maximum firing rate but that the neuron can be made to produce more spikes if the intensity of the stimuli are increased.

When producing or analyzing these different forms of data visualization, it is important to remember that the raw number of spikes that the investigator includes as data is determined by the settings on the electrophysiology rig and computer. For example, changing the voltage threshold that defines a spike will alter the number of spikes included in the data analysis.

Extracellular recordings do not require the precise and delicate electrode positioning that is necessary for intracellular or patch clamp recordings. However, a disadvantage of extracellular recordings is that it is impossible to measure graded, localized potentials within a neuron. Therefore, extracellular recordings cannot report detailed information about subthreshold potential changes, such as EPSPs or IPSPs. Instead, extracellular techniques are good at measuring information about action potentials detected just outside neurons of interest. Additionally, extracellular recordings can detect **local field potentials**, the sum of postsynaptic activity of multiple neurons within a volume of neural tissue, to determine activity changes in a population of neurons.

By using multiple electrodes, the activity of multiple cells can be recorded at the same time. A popular configuration is the use of tetrodes, a bundle of four individual electrodes carefully arranged into a single implant (Fig. 4.6A). Grids of individual electrodes or groups of tetrodes can be arranged into a single device called a multielectrode array (MEA) (Fig. 4.6B). This implant

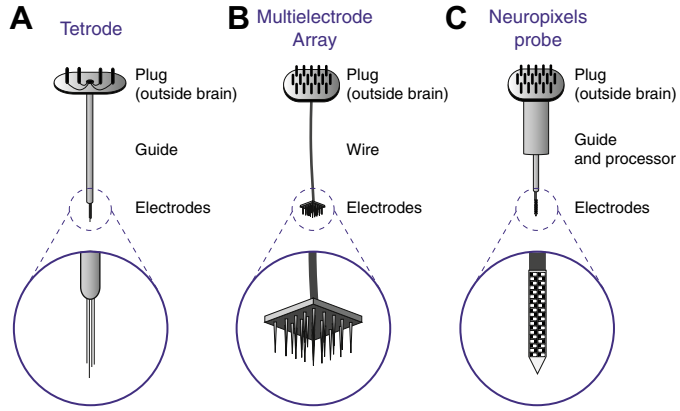


FIGURE 4.6 Specialized electrodes for multicellular recordings. (A) An individual tetrode is composed of four microelectrode wires. A scientist implants a tetrode into the brain of an animal and connects the top plug to a cable attached to an amplifier. (B) A multielectrode array is composed of 25 or more electrodes (sometimes over 100) and is used to record from neurons on the surface of the brain. Multielectrode arrays can also be used to record from slices in vitro. (C) A silicon neuropixels probe is composed of hundreds of individual recording sites arranged on an extremely thin shank.

allows for extracellular recording from several neighboring sites at once. The number of microelectrodes in an array varies, but can be anywhere from four (a single tetrode) to over 100.

Recent advances in **silicon probe** technology have greatly increased the number of neurons that researchers can record from simultaneously (Fig. 4.6C). These probes (better known as **Neuropixels**) allow researchers to increase their recording capacity due to the dense recording sites as well as a high channel count. Each probe contains 960 sites on a 10 mm long shank, and user-programmable switches allow recording channels to measure 384 of the 960 sites simultaneously. Using Neuropixels, researchers can visualize spiking activity from hundreds of neurons per probe implanted in mice, rats, or primates, a tremendous increase in the number of neurons that researchers can record from compared to traditional tetrodes or MEAs.

When recording from a single electrode, it can be difficult to determine whether the recorded electrical activity is produced by a single neuron or multiple neurons in the vicinity of the electrode tip. A single unit spike depends on the cell's unique shape, size, and distance from the recording electrode. **Spike sorting** is an analysis technique that can identify individual cells based on consistency in their activity patterns and assign waveforms to particular cells. A major advantage to recording from multiple channels at once is that it is easier to compare recordings made by each individual microelectrode. Signals are processed using triangulation methods based on the position of the electrodes and the strength and characteristic shape of a recording. These comparisons result in specific and reproducible waveforms

for each individual neuron recorded, enabling single unit data to be obtained by recording from multiple electrodes at once.

By investigating the activity of dozens to hundreds of neurons simultaneously, it is possible to answer questions regarding connectivity and timing within a neural network. A scientist can also manipulate activity in a population of neurons (Chapter 8) and monitor the effect on other neurons. Ultimately, using multiple electrodes to study the simultaneous responses of multiple neurons within circuits provides pivotal information about how neurons are connected and the spatiotemporal relationships of their activity.

Intracellular Recording

While extracellular recordings detect changes in membrane potential from outside of neurons, intracellular recordings can detect small, graded changes in membrane potential from within the cells themselves. An intracellular recording requires impaling a neuron or axon with a microelectrode to measure the potential difference between the tip of the intracellular electrode and the reference electrode positioned outside the cell.

The waveform of an action potential is different for extracellular and intracellular recordings (Fig. 4.5). From the point of view of an extracellular electrode, the difference in potential between the recording electrode and ground electrode initially decreases, then increases, and then returns to baseline. From the point of view of an intracellular electrode, the potential difference initially increases and then returns to baseline. Also notice that the units of measurement are different between the two techniques. Intracellular electrodes measure potential differences in units of millivolts (mV). Extracellular electrodes measure much smaller potential differences and are often expressed in units of microvolts (μV).

In the early days of electrophysiology, intracellular recordings were used to determine the ionic basis of membrane potential. In fact, Alan Hodgkin and Andrew Huxley won the Nobel Prize in 1963 for using intracellular methods to determine the ionic basis of action potentials. However, most experiments that once used intracellular electrodes are now usually performed using patch clamp techniques due to higher signal-to-noise ratios and the ability to ask questions about the nature of single ion channels. Indeed, after developing patch clamp techniques and using them to study the function of single ion channels, Erwin Neher and Bert Sakmann were awarded the Nobel Prize in 1991.

Patch Clamp Techniques

Using patch clamp methods, a glass micropipette is used to make tight contact with a tiny area, or patch, of neuronal membrane. After applying a small amount of suction to the back of the pipette, the seal between pipette and

membrane becomes so tight that ions can no longer flow between the pipette and the membrane. Thus, all the ions that flow when a single ion channel opens in the patched membrane must flow between the cell and the inside of the pipette. The resulting electrical current, though small, can be measured with a sensitive amplifier. This arrangement is commonly referred to as the **cell-attached mode** (Fig. 4.7). Resistance of the seal between the pipette and the cell membrane must be very high to ensure that all current flows through the pipette and does not leak out through other regions of the membrane. In practice, seal resistances in excess of 1 Gigaohm (GΩ) can be obtained by a cell-attached patch, often referred to as a "gigaseal."

While the cell-attached method allows an investigator to study single ion channels within a cell membrane, three other variations of the patch-clamp technique allow for measurement of other important physiological properties: whole-cell, inside-out, and outside-out recordings (Fig. 4.7). If the membrane patch within the pipette is disrupted by briefly applying strong suction, the interior of the pipette becomes continuous with the cytoplasm of the cell. This arrangement allows measurements of electrical potentials and currents from the entire cell and is therefore called the **whole-cell recording** method. The whole-cell configuration also allows diffusional exchange between the pipette and the cytoplasm, establishing a convenient way to inject substances into the interior of a patched cell. Two other variants of the patch clamp technique originate from the finding that once a tight seal has formed between the membrane and the glass pipette, small pieces of membrane can be pulled away from the cell without disrupting the seal (Fig. 4.7). Simply retracting a pipette that is in the cell-attached configuration causes a small vesicle of membrane to remain attached to the pipette. By exposing the tip of the pipette to air, the intracellular surface of a small patch of membrane is exposed. This arrangement, called the **inside-out recording** configuration, allows the measurement of single-channel currents with the added benefit of being able to change the medium exposed to the intracellular surface of the membrane. Thus, the inside-out configuration is particularly valuable when studying the influences of intracellular (cytoplasmic) molecules on ion channel function. Alternatively, if the pipette is retracted while it is in the whole-cell configuration, a membrane patch is produced that reseals to expose its extracellular surface to the bath solution. This arrangement, called the **outside-out recording** configuration, is optimal for studying how channel activity is influenced by extracellular chemical signals, such as neurotransmitters, because the bath solution can be easily manipulated during recordings. Table 4.2 compares the relative advantages and disadvantages of the four variations of the patch-clamp techniques.

Sometimes electrophysiologists use a fifth method: the perforated patch. The purpose of this technique is to make the glass pipette continuous with the cell, as in the whole-cell mode, but without the disadvantage of cytoplasmic contents potentially leaking into the pipette. Instead of applying suction in the

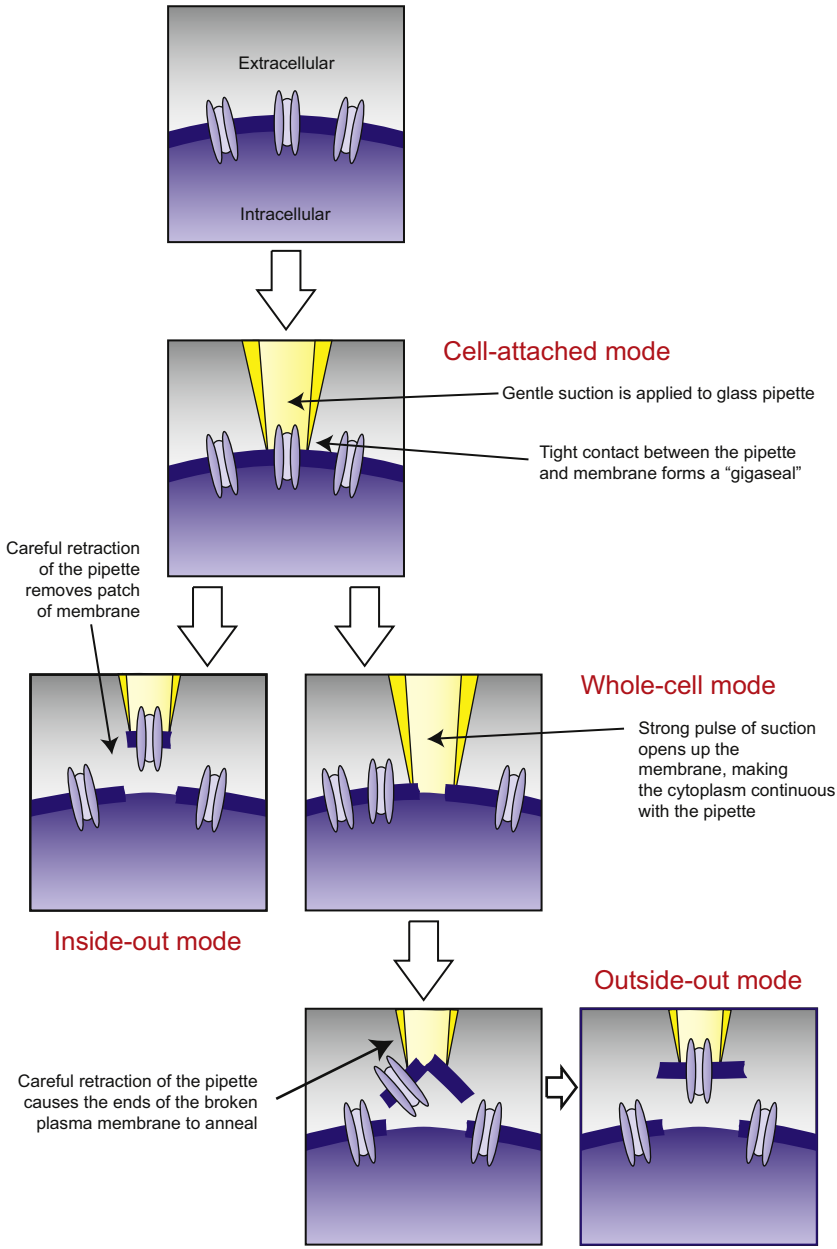


FIGURE 4.7 **The patch clamp techniques.** The four fundamental patch clamp techniques include the cell-attached mode, whole-cell mode, inside-out mode, and outside-out mode.

TABLE 4.2 Relative Advantages and Disadvantages of Patch Clamp Configurations.

	Advantages	Disadvantages
Cell-attached	Single ion channels can be recorded and channel properties are not changed. Physiologically relevant because cell is intact. Easiest patch clamp configuration to obtain.	Exact membrane potential of the patch is unknown. Difficult to manipulate the intracellular environment.
Whole-cell	Ability to manipulate the cell cytoplasm pharmacologically. The relatively large size of the current makes it easier to measure.	The cell is perforated, so cell contents may be diluted or leak out.
Excised patch (inside-out or outside-out)	Recordings can be taken from individual ion channels. Excellent environmental control over either the intracellular and extracellular sides of single ion channels.	There is a risk that channel properties are changed because the cellular environment is severely altered.

cell-attached mode to transition into the whole-cell mode, a chemical (often an antibiotic, such as amphotericin-B or nystatin) is applied from within the glass pipette, causing small perforations in the membrane. Thus, the perforated patch technique can be thought of as an intermediate between the cell-attached mode and whole-cell mode; this configuration prevents the leaking of cytoplasmic contents but generates a smaller signal than whole-cell mode.

An interesting technique called **dynamic clamp** allows scientists to simulate an artificial ion channel conductance or synaptic connection in living cells, even creating hybrid circuits between real and simulated neurons. In a dynamic clamp experiment, a cell's voltage is monitored, typically using whole-cell patch clamp, and fed into a computational model, either in the computer software or implemented through hardware as an analog chip. The model calculates and then sends back a current to inject into the cell, creating a real-time feedback loop. This applied current creates an experimental condition in which a channel with the model's properties seems as if it is embedded in the cell's membrane. Dynamic clamp can be used to simulate specific channel "knockins," artificial synaptic inputs, or virtual pharmacology experiments.

ELECTROPHYSIOLOGY TISSUE PREPARATIONS

In addition to the recording techniques described above, electrophysiology experiments can be also be categorized by the type of tissue preparation.

Physiological experiments in vitro usually fall into one of three main categories: recording from heterologous expression systems, primary cultured cells, or brain slices. It is also possible to perform experiments in vivo using extracellular techniques (and more rarely, intracellular and patch clamp techniques), either in an anesthetized animal or in an awake, behaving animal.

In Vitro Recordings

In vitro culture preparations provide unparalleled physical and visual access to individual cells, allowing detailed studies of the molecules and proteins that affect neuronal physiology. In these experiments, the neural specimen is incubated in a well-regulated culture media or bath solution. The composition of the solutions used during the experiments is critical because cellular electrical properties depend on the concentration gradient of ions inside and outside the cell. When deciding what ingredients should make up the bath solution, the guiding principle is to maintain an environment that allows the observation of physiologically relevant electrical activity and channel function. In patch clamp techniques, the solution inside the microelectrode should mimic the intracellular milieu. This solution is typically composed of salts, ionic buffering agents, and a pH buffer, which can be maintained at biologically relevant temperatures to provide cells and tissue with a simulated in vivo environment. One of the greatest advantages of in vitro recordings is the ability to study the electrophysiological properties of neurons by altering components of the bath. For example, to isolate the contribution of specific channels or receptors to the electrical signal, investigators frequently add pharmacological agents to the bath solution that block certain receptors, such as CNQX (to block AMPA receptors) or D-AP5 (to block NMDA receptors).

Heterologous Expression Systems

To study the physiological properties of individual ion channels, scientists often utilize **heterologous expression systems**. A heterologous expression system is a type of cell culture system (Chapter 13) that can be easily transfected with a foreign gene (Chapter 11), such as a gene that encodes a specific ion channel. The ion channel is efficiently expressed within the cells, and its function can be studied with methods such as the patch clamp techniques. By expressing mutant forms of a channel, scientists can observe the effect of molecular changes on a channel's electrical properties; for example, the role of a specific amino acid on a channel's opening and closing dynamics. Heterologous expression systems are also useful for isolating the role of individual channels because of the absence of endogenous channels that may otherwise alter overall cell physiology.

Common expression systems are *Xenopus laevis* oocytes, Chinese hamster ovary (CHO) cells, HELA cells, and human embryonic kidney (HEK 293T)

cells. These cells are usually easy to culture, readily available, have few endogenous channels, and can easily take up and express foreign DNA. The ability to combine molecular and physiological methods in a single cell system has made heterologous expression systems a powerful experimental tool for understanding the structure–function relationships of ion channels (Box 4.5).

BOX 4.5 Walkthrough of an In Vitro Electrophysiology Experiment

Let us say that you are interested in examining the properties of a newly discovered ion channel. Your research group has cloned the channel and created an expression construct (Chapter 10) and introduced this channel into *Xenopus laevis* oocytes (Chapter 11). We can now use this heterologous expression system, the large (~1 mm) *Xenopus* oocytes expressing our channel of interest, to answer questions about the electrical properties of the channel. You might be interested in a number of properties: What ions pass through the channel? How is the channel gated, and what stimuli open the channel to let ions pass? You might first look at the similarity of the amino acids to known channels. This can provide hypotheses about the properties of the channel, such as whether the channel is selective for specific ions or what types of compounds may affect gating properties. To examine single channel properties, patch clamp recordings will be the most appropriate technique.

Assuming the lab has established rigs on which to work, the first step in running the experiment will typically be to make the appropriate solutions and prepare fresh glass micropipettes. The composition of both the bath solution and the solution that will fill the micropipette is important because ionic concentration gradients affect the electrical properties of the membrane. The solution's osmolarity and pH will also affect pressure on the cell membrane, which can cause a cell to shrink or swell if not properly regulated. In a whole-cell recording, the pipette-filling solution will be continuous with the inside of the cell, so it should closely match the cell cytoplasm. The bath solution can contain pharmacological agents that block the activity of known channels so the signal from the channel under investigation stands out from background noise. However, currents of endogenous Xenopus ion channels are typically much smaller than those of heterologously overexpressed channels; thus, it is not always necessary to add blocking agents to the bath solution.

Patch pipettes are generally used immediately after they are made to ensure that the tip of the micropipette is not contaminated, which can cause a poor seal to form between the membrane and the pipette. A pulled glass pipette should be polished to round and smooth the tip. Now it is ready to be filled with solution and positioned in the electrode holder. Using the microscope and a micromanipulator, you can bring the electrode tip toward a targeted cell into the field of view. By applying gentle, positive pressure to the electrode through a tube to prevent dirtying the electrode tip, you can insert the tip into the bath solution. By applying a test voltage pulse, the patch electrode will generate a current, and you can calculate the electrode's resistance. Observing the current response to the test

Continued

BOX 4.5 Walkthrough of an In Vitro Electrophysiology Experiment—cont'd

pulse can help guide the electrode position. As the tip nears the cell, resistance should increase.

You can gently touch the electrode tip against the surface of the cell, checking the computer output of data to see that resistance has increased, and then release the positive pressure. This can be seen as a small dimple against the cell surface as the tip is pressed against the cell. You can now apply gentle suction to the patch pipette, using resistance and microscopic appearance as evidence that contact has been made between the cell surface and the patch electrode tip. If you have a gigaseal, you should see the resistance rise to at least 1 GΩ. Establishing the gigaseal is one of the trickiest parts of patch-clamping—perhaps the step that makes this technique an art.

At this point, you are in cell-attached mode and can switch the amplifier to voltage clamp. Cell-attached mode can provide single-channel resolution, and you may be able to see characteristic steps in the current that demonstrate transitions between the opened and closed state of a channel. Now you can test whether changes in voltage can activate the patched channel (or channels) by applying voltage steps. Alternatively, you can test whether the addition of ligands affect the patched channel by adding various compounds to the solution filling the micropipette (as this is the extracellular environment of the patched channel).

Monitoring the effect of experimental manipulations on current will reveal open/closed times and current amplitude. Current amplitude can be analyzed to determine the conductivity and ion specificity of the channel. I/V curves (Box 4.2) reveal characteristics of voltage-activated conductance, including the reversal potential, the voltage at which there is no overall flow of ions across the membrane. The reversal potential can be used to identify the ion species that pass through the channel.

Analyzing current flow through individual channels in this heterologous expression system exposes many of the channel's properties. Further studies can use mutant versions of the channel to dissect the requirements of specific aspects of the protein's structure on its function. This knowledge can then be used to investigate the contributions of this channel of interest in intact or natural preparations: isolated neurons, brain slices, and whole animals.

Primary Cultures

Using primary cultures of cells from the nervous system, a scientist can study electrophysiological properties of specific populations of neurons, but with better accessibility and visibility compared with recording from the same cells in vivo. Primary cultures are typically obtained from very young animals (Chapter 13), and some neural subtypes are easier to isolate and culture than others. These cultures are useful for comparing the electrophysiological properties of different populations of neurons, as well as studying the effects of

drugs or neurochemicals on membrane potentials. For example, an investigator may want to know what happens to a cell's membrane potential when the extracellular concentration of a particular ion or neuropeptide increases or decreases. An investigator can also compare the physiological properties of neurons from different populations of animals, for example, a population of neurons from a wild-type mouse versus a knockout mouse lacking a gene that encodes a specific receptor or ion channel. The disadvantage to using primary cultured cells is that, because they lack presynaptic input and their native environment within the nervous system, their normal electrophysiological properties may be highly altered.

Slice Cultures

The controlled in vitro environment in cell culture conditions can be substantially different from the in vivo environment. Brain slices preserve some endogenous connections while still providing the level of access available via cell culture conditions, more closely mimicking the in vivo environment. While it is possible to culture tissue slices for extended periods of time (Chapter 13), most brain slices are obtained and recorded in the same day. At the beginning of the experiment, the brain is removed and sliced into 300−500 μm thick sections using a vibratome (Chapter 6). Many neurons remain healthy despite the mechanical shock and damage of slicing, though physiological responses may be slightly altered. The brain slice is placed in a chamber that is continuously bathed in a solution containing the proper proportion of inorganic ions, nutrients, and gases to allow the neurons to survive.

There are several compelling advantages to recording from a brain slice rather than an intact brain. It is much easier to record from specific neurons if they are visible under a microscope. It is also easier to study neurons from a particular region of the brain if an electrode does not have to penetrate several millimeters of cells before it can get to that region. Brain slices provide much easier access to internal brain structures. In slice cultures, specific drugs or other pharmacological agents can easily be applied to a brain slice. Finally, the role of individual neurons in circuits can be studied because it is possible to record from both pre- and postsynaptic neurons in a known synaptic circuit (if they can be accessed within the same section).

In Vivo Recordings

Though in vitro recordings allow for a controlled environment and greater access to the brain, they may not accurately reflect neural activity in an intact organism. In vivo recordings allow a scientist to study neurons in their native environment within the nervous system. Additionally, neural activity can be correlated with animal behavior or other physiological phenotypes. These preparations usually require an investigator to perform an extracellular

recording because it is extremely difficult to visualize the cellular environment and to correctly position an electrode for intracellular or patch clamp recordings. However, it is possible to perform intracellular or even patch clamp recordings with specialized, dedicated equipment. To perform intracellular recordings, a scientist can use a very sharp pipette to impale cells for "sharp electrode" recordings. To perform in vivo patch clamp recordings, a scientist can use two-photon microscopy (Chapter 5) in an animal expressing a fluorescent protein in a cell type of interest to visualize the placement of the patch pipette on the surface of the membrane. A scientist can also perform patch clamp recordings "blind," but this approach is quite difficult and requires a great deal of serendipity in placing the electrode in a good location.

Acute Versus Chronic Recordings

While most in vitro recordings are performed during a single session, in vivo recordings can be performed either acutely (over hours) or chronically (over multiple days). An acute recording is an experiment that uses an animal only once. To perform a chronic recording, cannulae or electrodes can be surgically implanted into an animal's brain, permitting long-term access to a recording site (Chapter 3). Chronic recordings from implanted tetrodes, MEAs, or silicon probes permit the monitoring of neural circuit activity during complex behavioral tasks over long periods of time or during a variety of different conditions. Sealable chambers implanted on the skull (Chapter 3) allow an investigator to place new electrodes within the brain over periods of months in rodents, or years in nonhuman primates.

Anesthetized Versus Awake Animals

Some in vivo electrophysiology experiments record from animals stabilized under anesthesia. Anesthetized animals are immobile, allowing scientists to record neuronal responses to passive stimuli and characterize tuning properties of single neurons. Unfortunately, anesthesia affects neuronal excitability and normal neurotransmission, with varied effects depending on the anesthetic agent or neural population of interest. Thus, it is important for an investigator to know the specific effects of the anesthetic used to be able to properly interpret the data. For some experiments, the animals are also paralyzed to eliminate motion from breathing—in these instances, artificial, controlled ventilation is provided.

Recordings from awake, behaving animals are typically performed either by permanently implanting electrodes into the brain, or by implanting a sealable chamber (with cap) over the population of interest. This area is then sealed by implanting a chamber with a screwable cap (Chapter 3). After the animal has recovered from the surgery, electrophysiological recordings can be performed for a few hours each day. Single or multiunit recording during a

behavioral task allows scientists to examine the neural basis of various cognitive and behavioral phenomena.

CONCLUSION

Electrophysiology remains the most direct way to measure neural activity and study the molecular and cellular mechanisms that endow neurons with their electrical characteristics. A wide range of techniques and tissue preparations make it possible to record the activity of neurons in a dish, a slice, or an awake, behaving animal.

In the past 10–20 years, optical techniques have been developed that indirectly measure neural activity as a proxy for electrical activity—these techniques are the subject of Chapter 7. However, many scientists, even nonelectrophysiologists, consider electrophysiology techniques to be the backbone of neuroscience research—they are the most direct methods to investigate the electrical activity of neurons and the specific ions, receptors, channels, and signaling proteins that regulate their function.

SUGGESTED READING AND REFERENCES

Books

Boulton, A.A., Baker, G.B., Vanderwolf, C.H., 1990. Neurophysiological Techniques: Applications to Neural Systems. Humana Press.

Covey, E., Carter, M.E. (Eds.), 2015. Basic Electrophysiology Methods. Oxford University Press.

Hille, B., 2001. Ion Channels of Excitable Membranes, third ed. Sinauer Associates, Sunderland, MA.

Molleman, A., 2003. Patch Clamping: An Introductory Guide to Patch Clamp Electrophysiology. Wiley.

Martin, A.R., Brown, D.A., Diamond, M.E., Cattaneo, A., De-Miguel, F.F., 2020. From Neuron to Brain, sixth ed. Sinauer Associates.

Nicolelis, M.A.L., 2008. Methods for Neural Ensemble Recordings, second ed. CRC Press, Boca Raton, FL.

Rettinger, J., Schwarz, S., Schwarz, S., 2016. Electrophysiology: Basics, Modern Approaches, and Applications. Springer.

The Axon Guide: A Guide to Electrophysiology & Biophysics Laboratory Techniques, fourth ed., 2020. Molecular Devices/MDS Analytical Technologies.

Review Articles

Buzsaki, G., 2004. Large-scale recording of neuronal ensembles. Nat. Neurosci. 7, 446–451.

Cunningham, J.P., Yu, B.M., 2014. Dimensionality reduction for large-scale neural recordings. Nat. Neurosci.

Economo, M.N., Fernandez, F.R., White, J.A., 2010. Dynamic clamp: alteration of response properties and creation of virtual realities in neurophysiology. J. Neurosci. 30, 2407–2413.

Hong, G., Lieber, C.M., 2019. Novel electrode technologies for neural recordings. Nat. Rev. Neurosci. 20, 330–345.

Miller, E.K., Wilson, M.A., 2008. All my circuits: using multiple electrodes to understand functioning neural networks. Neuron 60, 483–488.

Quian Quiroga, R., Panzeri, S., 2009. Extracting information from neuronal populations: information theory and decoding approaches. Nat. Rev. Neurosci. 10, 173–185.

Super, H., Roelfsema, P.R., 2005. Chronic multiunit recordings in behaving animals: advantages and limitations. Prog. Brain Res. 147, 263–282.

Windels, F., 2006. Neuronal activity: from in vitro preparation to behaving animals. Mol. Neurobiol. 34, 1–26.

Primary Research Articles—Interesting Examples from the Literature

Allen, W.E., Chen, M.Z., Pichamoorthy, N., Tien, R.H., Pachitariu, M., Luo, L., Deisseroth, K., 2019. Thirst regulates motivated behavior through modulation of brainwide neural population dynamics. Science 364, 253.

Bennett, C., Arroyo, S., Hestrin, S., 2013. Subthreshold mechanisms underlying state-dependent modulation of visual responses. Neuron 80, 350–357.

Evarts, E.V., 1968. A technique for recording activity of subcortical neurons in moving animals. Electroencephalogr. Clin. Neurophysiol. 24, 83–86.

Foster, D.J., Wilson, M.A., 2006. Reverse replay of behavioural sequences in hippocampal place cells during the awake state. Nature 440, 603–680.

Jun, J.J., et al., 2017. Fully integrated silicon probes for high-density recording of neural activity. Nature 551, 232–236.

Kaufman, M.T., Churchland, M.M., Ryu, S.I., Shenoy, K.V., 2014. Cortical activity in the null space: permitting preparation without movement. Nat. Neurosci. 17, 440–448.

Kisner, A., Slocomb, J.E., Sarsfield, S., Zuccoli, M.L., Siemian, J., Gupta, J.F., Kumar, A., Aponte, Y., 2018. Electrophysiological properties and projections of lateral hypothalamic parvalbumin positive neurons. PLoS One 13, e0198991.

Lin, D., Boyle, M.P., Dollar, P., Lee, H., Lein, E.S., Perona, P., Anderson, D.J., 2011. Functional identification of an aggression locus in the mouse hypothalamus. Nature 470, 221–226.

Mante, V., Sussillo, D., Shenoy, K.V., Newsome, W.T., 2013. Context-dependent computation by recurrent dynamics in prefrontal cortex. Nature 503, 78–84.

Sharp, A.A., O'Neil, M.B., Abbott, L.F., Marder, E., 1993. Dynamic clamp: computer-generated conductances in real neurons. J. Neurophysiol. 69, 992–995.

Steinmetz, N.A., et al., 2021. Neuropixels 2.0: a miniaturized high-density probe for stable, long-term brain recordings. Science 372, eabf4588.

Protocols

Brown, A.L., Johnson, B.E., Goodman, M.B., 2008. Patch clamp recording of ion channels expressed in Xenopus oocytes. J. Vis. Exp. 20. https://doi.org/10.3791/936. http://www.jove.com/index/details.stp?id=936.

Current Protocols in Neuroscience, 2007. Chapter 6: Neurophysiology. John Wiley & Sons, Inc.

Kodandaramaiah, S.B., Franzesi, G.T., Chow, B.Y., Boyden, E.S., Forest, C.R., 2012. Automated whole-cell patch-clamp electrophysiology of neurons *in vivo*. Nat. Methods 9, 585–587.

Martina, M., Taverna, S. (Eds.), 2014. Patch-Clamp Methods and Protocols (Methods in Molecular Biology), second ed. Humana Press, Totowa, NJ.

Murthy, M., Turner, G., 2013. Whole-cell in vivo patch-clamp recordings in the Drosophila brain. Cold Spring Harb. Protoc. 2013 (2), 140–148.

Nicolelis, M.A., Dimitrov, D., Carmena, J.M., Crist, R., Lehew, G., Kralik, J.D., Wise, S.P., 2003. Chronic, multisite, multielectrode recordings in macaque monkeys. Proc. Natl. Acad. Sci. U. S. A. 100, 11041–11046.

Perkins, K.L., 2006. Cell-attached voltage-clamp and current-clamp recording and stimulation techniques in brain slices. J. Neurosci. Methods 154, 1–18.

Tammaro, P., Shimomura, K., Proks, P., 2008. Xenopus oocytes as a heterologous expression system for studying ion channels with the patch-clamp technique. Methods Mol. Biol. 491, 127–139.