BIOELECTRICITY

This chapter introduces the basic concepts used in making electrical measurements from cells and in describing instruments used in making these measurements.

Electrical Potentials

A cell derives its electrical properties mostly from the electrical properties of its membrane. A membrane, in turn, acquires its properties from its lipids and proteins, such as ion channels and transporters. An *electrical potential difference* exists between the interior and exterior of cells. A charged object (ion) gains or loses energy as it moves between places of different electrical potential, just as an object with mass moves "up" or "down" between points of different gravitational potential. Electrical potential differences are usually denoted as V or ΔV and measured in *volts*; therefore, potential is also termed *voltage*. The potential difference across a cell relates the potential of the cell's interior to that of the external solution, which, according to the commonly accepted convention, is zero.

Potential differences between two points that are separated by an insulator are larger than the differences between these points separated by a conductor. Thus, the lipid membrane, which is a good insulator, has an electrical potential difference across it. This potential difference ("transmembrane potential") amounts to less than 0.1 V, typically 30 to 90 mV in most animal cells, but can be as much as 150 - 200 mV in plant cells. On the other hand, the salt-rich solutions of the cytoplasm and blood are fairly good conductors, and there are usually very small differences at steady state (rarely more than a few millivolts) between any two points within a cell's cytoplasm or within the extracellular solution. Electrophysiological equipment enables researchers to measure potential (voltage) differences in biological systems.
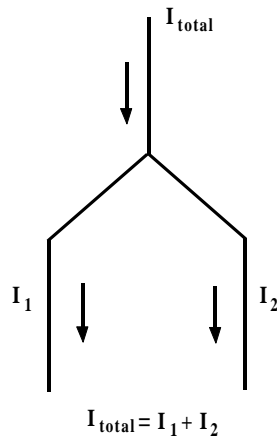
Electrical Currents

Electrophysiological equipment can also measure current, which is the flow of electrical charge passing a point per unit of time. Current (I) is measured in amperes (A). Usually, currents measured by electrophysiological equipment range from picoamperes to microamperes. For

instance, typically, 10^4 Na^+ ions cross the membrane each millisecond that a single Na^+ channel is open. This current equals 1.6 pA (1.6 x 10^{-19} coul/ion x 10^4 ions/ms x 10^3 ms/s).

Two handy rules about currents often help to understand electrophysiological phenomena: (1) current is conserved at a branch point (Figure 1-1); and (2) current always flows in a complete circuit (Figure 1-2). In electrophysiological measurements, currents can flow through capacitors, resistors, ion channels, amplifiers, electrodes and other entities, but they always flow in complete circuits.

$$I_{total} = I_1 + I_2$$

Figure 1-1. Conservation of Current

Current is conserved at a branch point.

$$I = I_1 + I_2$$
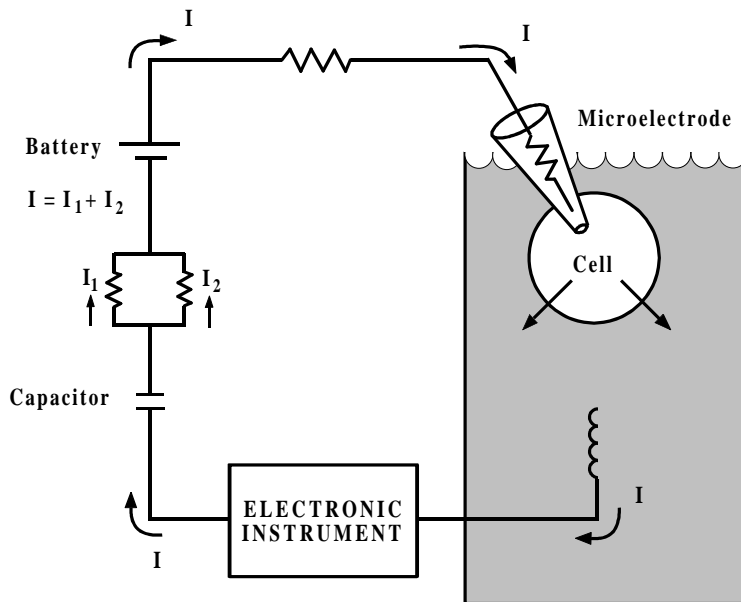
Figure 1-2. A Typical Electrical Circuit

Example of an electrical circuit with various parts. Current always flows in a complete circuit.

Resistors and Conductors

Currents flow through *resistors* or *conductors*. The two terms actually complement one another — the former emphasizes the barriers to current flow, while the latter emphasizes the pathways for flow. In quantitative terms, resistance R (units: ohms (Ω)) is the inverse of conductance G (units: siemens (S)); thus, infinite resistance is zero conductance. In electrophysiology, it is convenient to discuss currents in terms of conductance because side-by-side ("parallel") conductances simply summate (Figure 1-3). The most important application of the parallel conductances involves ion channels. When several ion channels in a membrane are open simultaneously, the total conductance is simply the sum of the conductances of the individual open channels.
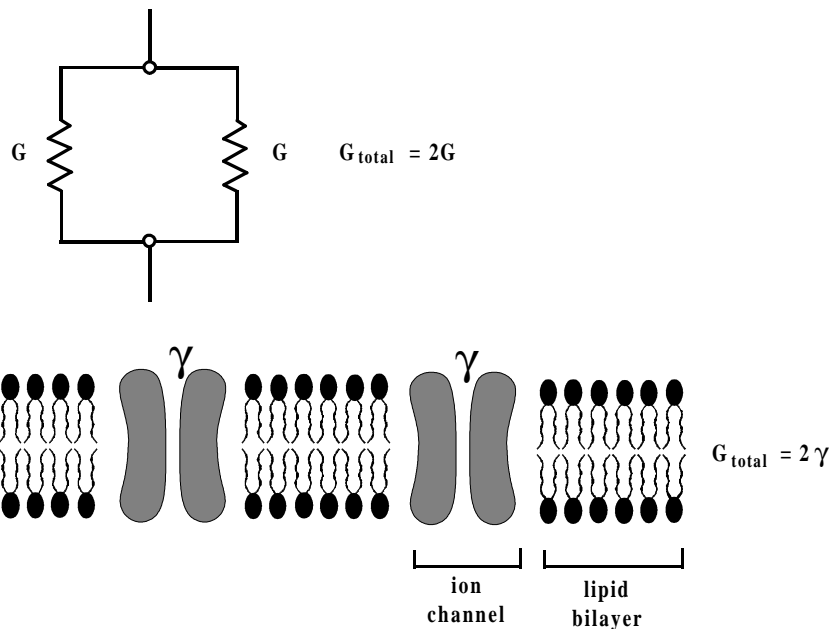
Figure 1-3. Summation of Conductance

Conductances in parallel summate together, whether they are resistors or channels.

A more accurate representation of an ion channel is a conductor in series with two additional circuit elements (Figure 1-4): (1) a switch that represents the gate of the channel, which would be in its conducting position when the gate is open, and (2) a battery that represents the *reversal potential* of the ionic current for that channel. The reversal potential is defined operationally as the voltage at which the current changes its direction. For a perfectly selective channel (*i.e.*, a channel through which only a single type of ion can pass), the reversal potential equals the *Nernst potential* for the permeant ion. The Nernst potential for ion A, E_a, can be calculated by the Nernst equation:

$$E_A = (RT/z_AF)\ln\{[A]_o/[A]_i\} = 2.303(RT/z_AF)\log_{10}\{[A]_o/[A]_i\} \quad \text{(units: volts)} \qquad (1)$$

where R is the gas constant (8.314 V C K^{-1} mol^{-1}), T is the absolute temperature (T = 273° + C°), z_A is the charge of ion A, F is Faraday's constant (9.648x10^4 C mol^{-1}), and $[A]_o$ and $[A]_i$ are the

concentrations of ion A outside the cell and inside the cell, respectively. At 20°C ("room temperature"), $2.303(RT/z_A F) = 58$ mV for a univalent ion.
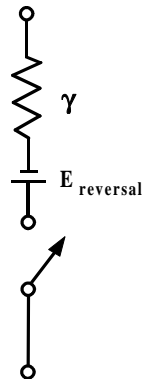
Figure 1-4. Equivalent Circuit for a Single-Membrane Channel

A more realistic equivalent circuit for a single-membrane channel.

For instance, at room temperature, a Na^+ channel facing intracellular Na^+ concentration that is ten-fold lower than the extracellular concentration of this ion would be represented by a battery of +58 mV. A K^+ channel, for which the concentration gradient is usually reversed, would be represented by a battery of -58 mV.

Reversal potentials are not easily predicted for channels that are permeable to more than one ion. Nonspecific cation channels, such as nicotinic acetylcholine receptors, usually have reversal potentials near zero millivolts. Furthermore, many open channels have a nonlinear relation between current and voltage. Consequently, representing channels as resistors is only an approximation. Considerable biophysical research has been devoted to understanding the current-voltage relations of ion channels and how they are affected by the properties and concentrations of permeant ions.

The transmembrane potential is defined as the potential at the inner side of the membrane relative to the potential at the outer side of the membrane. The *resting membrane potential* (E_{rp}) describes a steady-state condition with no net flow of electrical current across the membrane. The resting membrane potential is determined by the intracellular and extracellular concentrations of ions to which the membrane is permeable and on their permeabilities. If one ionic conductance is dominant, the resting potential is near the Nernst potential for that ion. Since a typical cell membrane at rest has a much higher permeability to potassium (P_K) than to sodium, calcium or chloride (P_{Na}, P_{Ca} and P_{Cl}, respectively), the resting membrane potential is very close to E_K, the potassium reversal potential.

Ohm's Law

For electrophysiology, perhaps the most important law of electricity is Ohm's law. The potential difference between two points linked by a current path with a conductance G and a current I (Figure 1-5) is:

$$\Delta V = IR = I/G \ \text{(units: volts)} \tag{2}$$
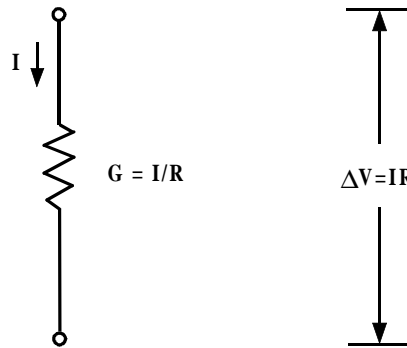
Figure 1-5. Ohm's Law

This concept applies to any electrophysiological measurement, as illustrated by the two following examples: (1) In an extracellular recording experiment: the current I that flows between parts of a cell through the external resistance R produces a potential difference ΔV, which is usually less than 1 mV (Figure 1-6). As the impulse propagates, I changes and, therefore, ΔV changes as well.
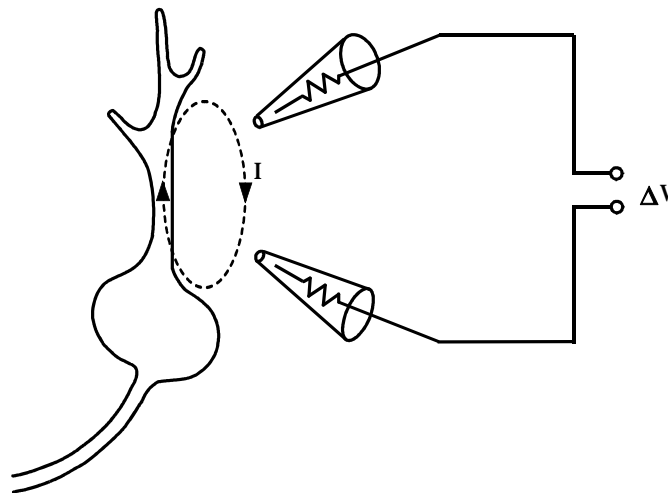
Figure 1-6. IR Drop

In extracellular recording, current I that flows between points of a cell is measured as the potential difference ("IR drop") across the resistance R of the fluid between the two electrodes.

(2) In a voltage-clamp experiment: when N channels, each of conductance γ, are open, the total conductance is $N\gamma$. The electrochemical driving force ΔV (membrane potential minus reversal potential) produces a current $N\gamma\Delta V$. As channels open and close, N changes and so does the voltage-clamp current I. Hence, the voltage-clamp current is simply proportional to the number of open channels at any given time. Each channel can be considered as a γ conductance increment.

The Voltage Divider

Figure 1-7 describes a simple circuit called a *voltage divider* in which two resistors are connected in series:
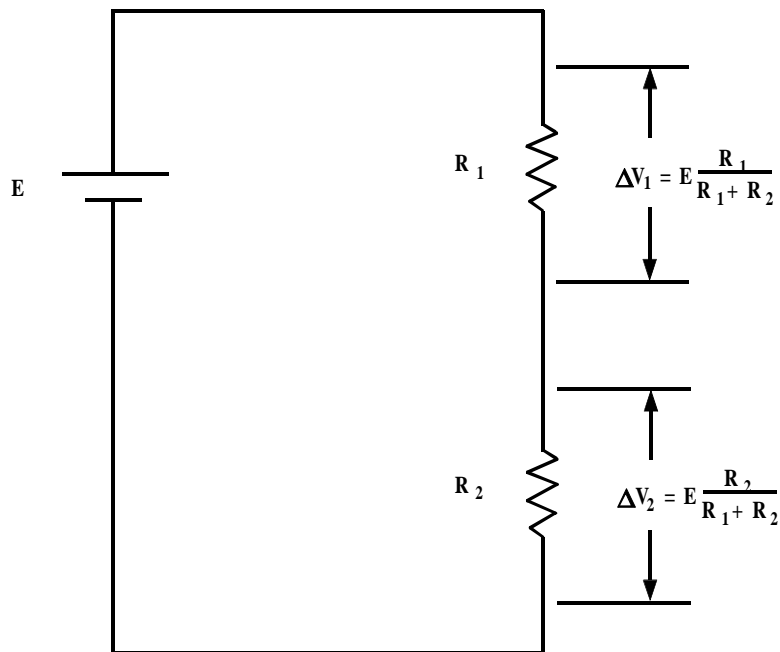
Figure 1-7. A Voltage Divider

The total potential difference provided by the battery is E; a portion of this voltage appears across each resistor.

When two resistors are connected in series, the same current passes through each of them. Therefore the circuit is described by

$$\Delta V_1 = E\frac{R_1}{R_1 + R_2} \quad ; \quad \Delta V_2 = E\frac{R_2}{R_1 + R_2} \tag{3a}$$
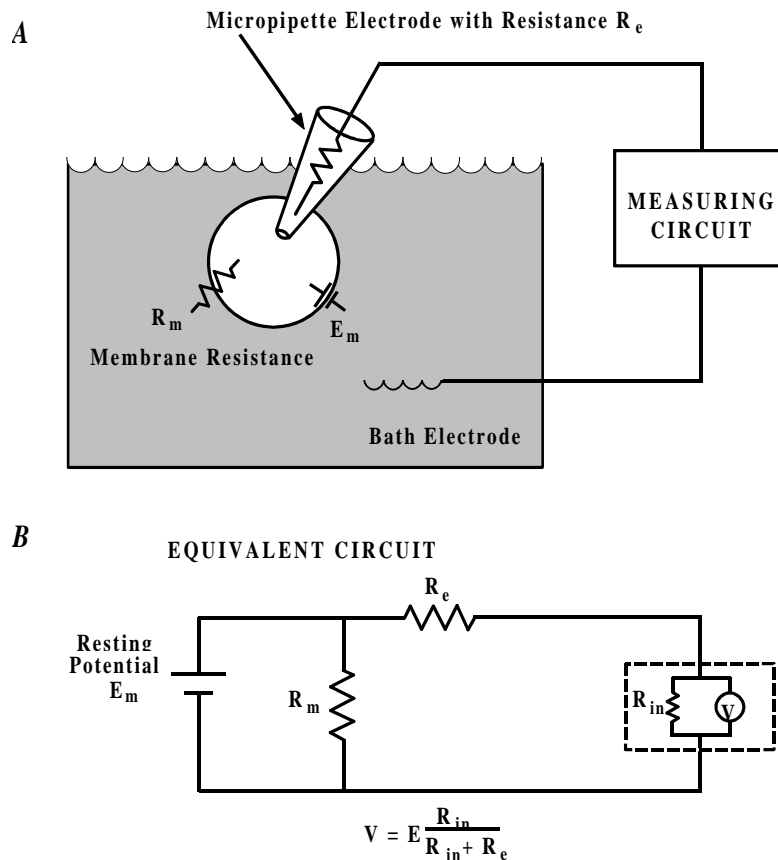
$$\Delta V_1 + \Delta V_2 = E \tag{3b}$$

where E is the value of the battery, which equals the total potential difference across both resistors. As a result, the potential difference is divided in proportion to the two resistance values.

Perfect and Real Electrical Instruments

Electrophysiological measurements should satisfy two requirements: (1) They should accurately measure the parameter of interest, and (2) they should produce no perturbation of the parameter. The first requirement can be discussed in terms of a voltage divider. The second point will be discussed after addressing electrodes.

The best way to measure an electrical potential difference is to use a voltmeter with infinite resistance. To illustrate this point, consider the arrangement of Figure 1-8(*A*), which can be reduced to the *equivalent circuit* of Figure 1-8(*B*).

A

Micropipette Electrode with Resistance R_e

MEASURING CIRCUIT

R_m

E_m

Membrane Resistance

Bath Electrode

B

EQUIVALENT CIRCUIT

R_e

Resting Potential E_m

R_m

R_{in}

$$V = E \frac{R_{in}}{R_{in} + R_e}$$

Figure 1-8. Representative Voltmeter with Infinite Resistance

Instruments used to measure potentials must have a very high input resistance R_{in}.

Before making the measurement, the cell has a resting potential of E_{rp}, which is to be measured with an intracellular electrode of resistance R_e. To understand the effect of the measuring circuit on the measured parameter, we will pretend that our instrument is a "perfect" voltmeter (*i.e.,* with an infinite resistance) in parallel with a finite resistance R_{in}, which represents the resistance of a real voltmeter or the measuring circuit. The combination R_e and R_{in} forms a voltage divider, so that only a fraction of E_{rp} appears at the input of the "perfect" voltmeter; this fraction equals $E_{rp}R_{in}/(R_{in} + R_e)$. The larger R_{in}, the closer V is to E_{rp}. Clearly the problem gets more serious as the electrode resistance R_e increases, but the best solution is to make R_{in} as large as possible.

On the other hand, the best way to measure current is to open the path and insert an ammeter. If the ammeter has zero resistance, it will not perturb the circuit since there is no IR-drop across it.

Ions in Solutions and Electrodes

Ohm's law — the linear relation between potential difference and current flow — applies to aqueous ionic solutions, such as blood, cytoplasm and sea water. Complications are introduced by two factors:
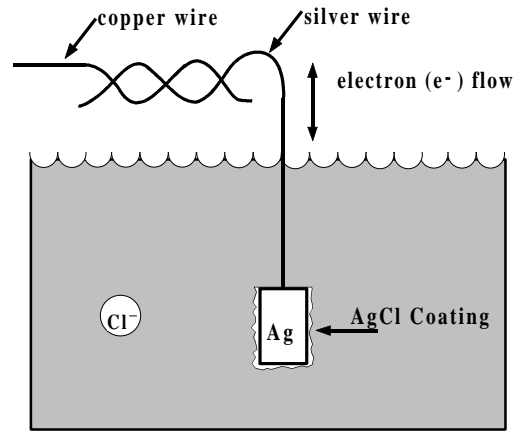
(1) The current is carried by at least two types of ions (one anion and one cation) and often by many more. For each ion, current flow *in the bulk solution* is proportional to the potential difference. For a first approximation, the conductance of the whole solution is simply the sum of the conductances contributed by each ionic species. When the current flows through ion channels, it is carried selectively by only a subset of the ions in the solution.

(2) At the electrodes, current must be transformed smoothly from a flow of electrons in the copper wire to a flow of ions in solution. Many sources of errors (artifacts) are possible. Several types of electrodes are used in electrophysiological measurements; the most common is a silver/silver chloride (Ag/AgCl) interface, which is a silver wire coated with silver chloride (Figure 1-9). If electrons flow from the copper wire through the silver wire to the electrode AgCl pellet, they convert the AgCl to Ag atoms and the Cl^- ions become hydrated and enter the solution. If electrons flow in the reverse direction, Ag atoms in the silver wire that is coated with AgCl give up their electrons (one electron per atom) and combine with Cl^- ions that are in the solution to make insoluble AgCl. This is, therefore, a *reversible electrode*, *i.e.*, current can flow in both directions. There are several points to remember about Ag/AgCl electrodes: (1) The Ag/AgCl electrode performs well only in solutions containing chloride ions; (2) Because current must flow in a complete circuit, two electrodes are needed. If the two electrodes face different Cl^- concentrations (for instance, 3 M KCl inside a micropipette[*] and 120 mM NaCl in a bathing solution surrounding the cell), there will be a difference in the half-cell potentials (the potential difference between the solution and the electrode) at the two electrodes, resulting in a large steady potential difference in the two wires attached to the electrodes. This steady potential difference, termed *liquid junction* potential, can be subtracted electronically and poses few problems as long as the electrode is used within its reversible limits; (3) If the AgCl is exhausted by the current flow, bare silver could come in contact with the solution. Silver ions leaking from the wire can poison many proteins. Also, the half-cell potentials now become dominated by unpredictable, poorly reversible surface reactions due to other ions in the solution and trace impurities in the silver, causing electrode *polarization*. However, used properly, Ag/AgCl electrodes possess the advantages of little polarization and predictable junction potential.
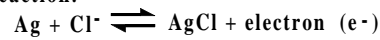
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[*] A micropipette is a pulled capillary glass into which the Ag/AgCl electrode is inserted (see **Chapter 4**).
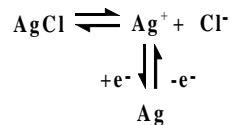
Electrode reaction:

$$Ag + Cl^- \rightleftharpoons AgCl + electron\ (e^-)$$

This reaction can also be presented by:

$$AgCl \rightleftharpoons Ag^+ + Cl^-$$

$$+e^- \updownarrow -e^-$$

$$Ag$$

Figure 1-9. The Silver/Silver Chloride Electrode

The silver/silver chloride electrode is reversible but exhaustible.

Another type of electrode, made of platinum (Pt) (Figure 1-10), is irreversible but not exhaustible. At its surface, Pt catalyzes the electrolysis of water. The gaseous H_2 or O_2 produced, depending on the direction of current flow, leaves the surface of the electrode. If both electrodes are Pt electrodes, the hydroxyl ions and protons are produced in equal numbers; however, *local* pH changes can still occur.

$$e^- + H_2O \longrightarrow OH^- + \tfrac{1}{2}H_2 \quad or \quad H_2O \longrightarrow 2H^+ + \tfrac{1}{2}O_2 + e^-$$
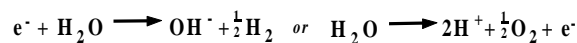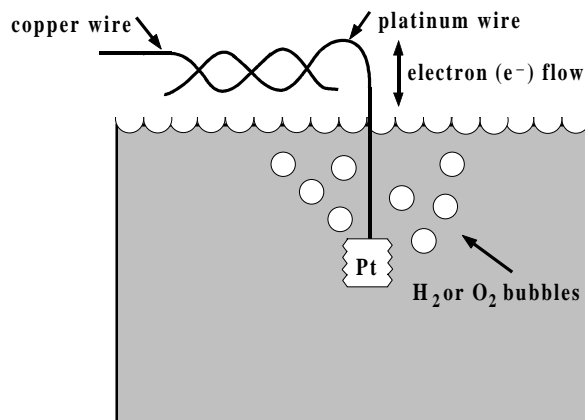
Figure 1-10. The Platinum Electrode

A platinum electrode is irreversible but inexhaustible.

Capacitors and Their Electrical Fields

The *electrical field* is a property of each point in space and is defined as proportional to the force experienced by a charge placed at that point. The greater the potential difference between two points fixed in space, the greater the field at each point between them. Formally, the electrical field is a vector defined as the negative of the spatial derivative of the potential.

The concept of the electrical field is important for understanding membrane function. Biological membranes are typically less than 10 nm thick. Consequently, a transmembrane resting potential of about 100 mV produces a very sizable electrical field in the membrane of about 10^5 V/cm. This is close to the value at which most insulators break down irreversibly because their atoms become ionized. Of course, typical electrophysiological equipment cannot measure these fields directly. However, changes in these fields are presumably sensed by the *gating domains* of voltage-sensitive ion channels, which determine the opening and closing of channels, and so the electrical fields underlie the electrical excitability of membranes.

Another consequence of the membrane's thinness is that it makes an excellent *capacitor*. Capacitance (C; measured in farads, F) is the ability to store charge Q when a voltage ΔV occurs across the two "ends," so that

$$Q = C\Delta V \tag{4}$$

The formal symbol for a capacitor is two parallel lines (Figure 1-2). This symbol arose because the most effective capacitors are parallel conducting plates of large area separated by a thin sheet of insulator (Figure 1-11) — an excellent approximation of the lipid bilayer. The capacitance C is proportional to the area and inversely proportional to the distance separating the two conducting sheets.
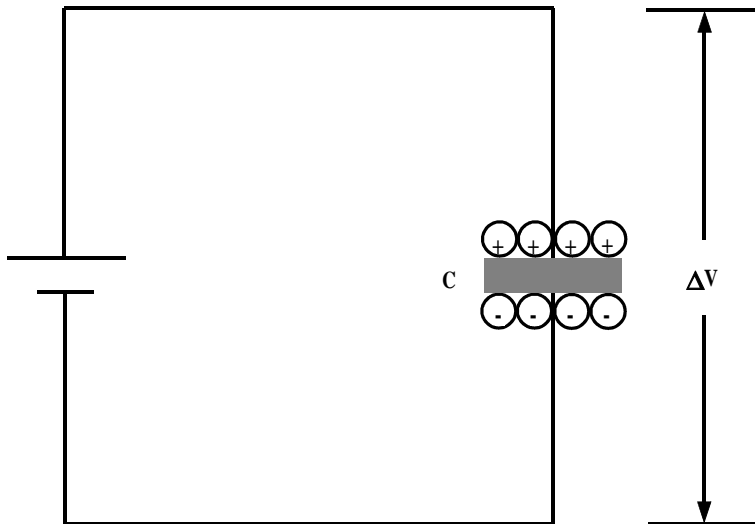

Figure 1-11. Capacitance
A charge Q is stored in a capacitor of value C held at a potential ΔV.

When multiple capacitors are connected in parallel, this is electronically equivalent to a single large capacitor; that is, the total capacitance is the sum of their individual capacitance values (Figure 1-12). Thus, membrane capacitance increases with cell size. Membrane capacitance is usually expressed as value per unit area; nearly all lipid bilayer membranes of cells have a capacitance of 1 $\mu F/cm^2$ (0.01 $pF/\mu m^2$).
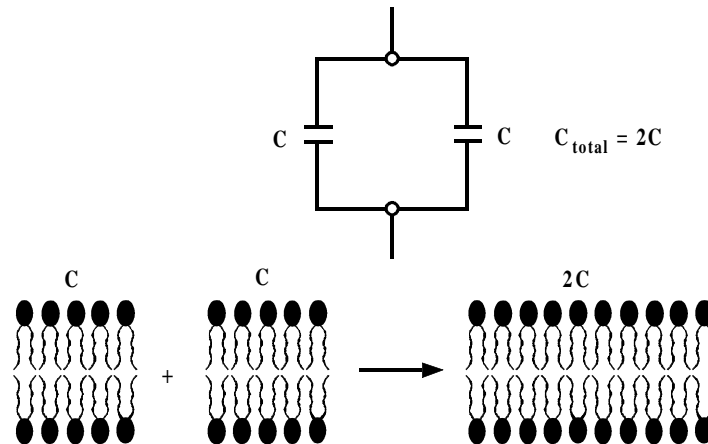
Figure 1-12. Capacitors in Parallel Add Their Values

Currents Through Capacitors

Equation 4 shows that charge is stored in a capacitor only when there is a change in the voltage across the capacitor. Therefore, the current flowing through capacitance C is proportional to the voltage change with time:

$$I = C\frac{\Delta V}{\Delta t} \tag{5}$$

Until now, we have been discussing circuits whose properties do not change with time. As long as the voltage across a membrane remains constant, one can ignore the effect of the membrane capacitance on the currents flowing across the membrane through ion channels. While the voltage changes, there are transient *capacitive currents* in addition to the steady-state currents through conductive channels. These capacitive currents constitute one of the two major influences on the time-dependent electrical properties of cells (the other is the kinetics of channel gating). On Axon Instruments voltage- or patch-clamp amplifiers, several controls are devoted to handle these capacitive currents. Therefore it is worth obtaining some intuitive "feel" for their behavior.

The stored charge on the membrane capacitance *accompanies* the resting potential, and any change in the voltage across the membrane is accompanied by a change in this stored charge. Indeed, if a current is applied to the membrane, either by channels elsewhere in the cell or by current from the electrode, this current *first satisfies the requirement for charging the membrane capacitance, then it changes the membrane voltage*. Formally, this can be shown by representing the membrane as a resistor of value R in parallel with capacitance C (Figure 1-13):
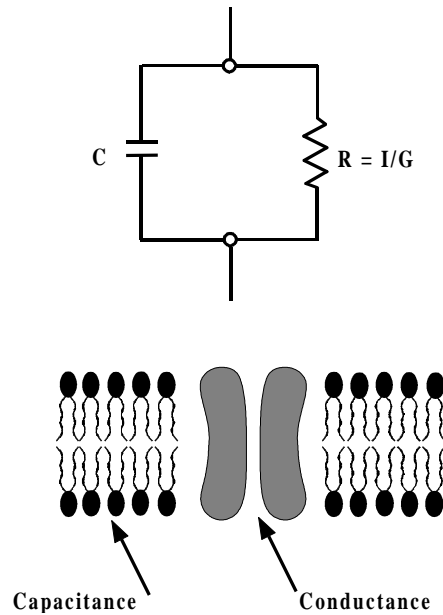
Figure 1-13. Membrane Behavior Compared with an Electrical Current

A membrane behaves electrically like a capacitance in parallel with a resistance.

Now, if we apply a pulse of current to the circuit, the current first charges up the capacitance, then changes the voltage (Figure 1-14).
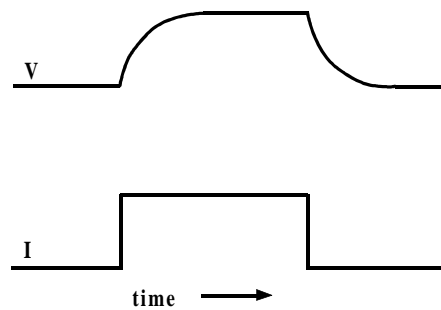
Figure 1-14. RC Parallel Circuit Response

Response of an RC parallel circuit to a step of current.

The voltage V(t) approaches steady state along an exponential time course:

$$V(t) = V_{inf}(1 - e^{-t/\tau}) \tag{5}$$

The steady-state value V_{inf} (also called the infinite-time or equilibrium value) does not depend on the capacitance; it is simply determined by the current I and the membrane resistance R:

$$V_{inf} = IR \tag{6}$$

This is just Ohm's law, of course; but when the membrane capacitance is in the circuit, the voltage is not reached immediately. Instead, it is approached with the *time constant* τ, given by

$$\tau = RC \tag{7}$$

Thus, the charging time constant increases when either the membrane capacitance or the resistance increases. Consequently, large cells, such as *Xenopus* oocytes that are frequently used for expression of genes encoding ion-channel proteins, and cells with extensive membrane invigorations, such as the T-system in skeletal muscle, have a long charging phase.

Current Clamp and Voltage Clamp

In a current-clamp experiment, one applies a known constant or time-varying current and measures the change in membrane potential caused by the applied current. This type of experiment mimics the current produced by a synaptic input.

In a voltage clamp experiment one controls the membrane voltage and measures the transmembrane current required to maintain that voltage. Despite the fact that voltage clamp does not mimic a process found in nature, there are three reasons to do such an experiment: (1) Clamping the voltage eliminates the capacitive current, except for a brief time following a step to a new voltage (Figure 1-15). The brevity of the capacitive current depends on many factors that are discussed in following chapters; (2) Except for the brief charging time, the currents that flow are proportional only to the membrane conductance, *i.e.*, to the number of open channels; (3) If channel gating is determined by the transmembrane voltage alone (and is insensitive to other parameters such as the current and the history of the voltage), voltage clamp offers control over the key variable that determines the opening and closing of ion channels.
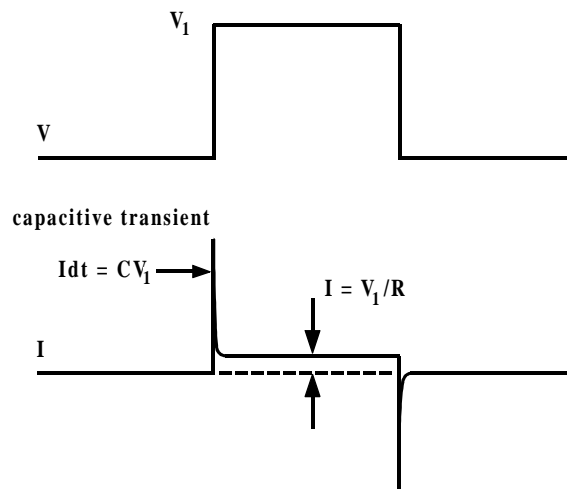
Figure 1-15. Typical Voltage-Clamp Experiment

A voltage-clamp experiment on the circuit of Figure 1-13.

The *patch clamp* is a special voltage clamp that allows one to resolve currents flowing through single ion channels. It also simplified the measurement of currents flowing through the whole-cell membrane, particularly in small cells that cannot be easily penetrated with electrodes. The characteristics of a patch clamp are dictated by two facts: (1) The currents measured are very small, on the order of picoamperes in single-channel recording and usually up to several nanoamperes in whole-cell recording. Due to the small currents, particularly in single-channel recording, the electrode polarizations and nonlinearities are negligible and the Ag/AgCl electrode can record voltage accurately even while passing current. (2) The electronic ammeter must be carefully designed to avoid adding appreciable noise to the currents it measures.

Glass Microelectrodes and Tight Seals

Successful electrophysiological measurements depend on two technologies: the design of electronic instrumentation and the properties and fabrication of glass micropipettes. Glass pipettes are used both for intracellular recording and for patch recording; recently quartz pipettes were used for ultra low-noise single-channel recording (for a detailed discussion of electrode glasses see **Chapter 4**). Successful patch recording requires a tight seal between the pipette and the membrane. Although there is not yet a satisfactory molecular description of this seal, we can describe its electrical characteristics.

Requirement (3) above (under *Perfect and Real Electrical Instruments*) states that the quality of the measurement depends on minimizing perturbation of the cells. For the case of voltage recording, this point can be presented with the voltage divider circuit (Figure 1-16).

EQUIVALENT CIRCUIT

$$V = E_K \frac{R_S}{R_S + R_K}$$
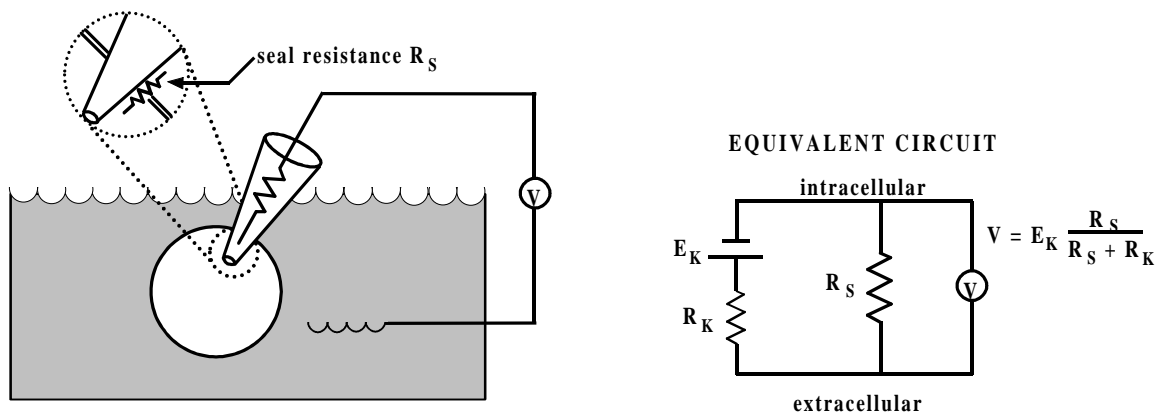
Figure 1-16. Intracellular Electrode Measurement

This intracellular electrode is measuring the resting potential of a cell whose membrane contains only open K^+ channels. As the seal resistance R_S increases, the measurement approaches the value of E_K.

For the case of patch recording, currents through the seal do not distort the measured voltage or current, but they do add to the current noise. Current noise can be analyzed either in terms of the *Johnson noise* of a conductor, which is the thermal noise that increases with the conductance (see **Chapter 7** and **Chapter 12**), or in terms of simple statistics. The latter goes as follows: If a current of N ions/ms passes through an open channel, then the current will fluctuate from one

millisecond to the next with a standard deviation of $\sqrt{N}$. These fluctuations produce noise on the single-channel recorded traces. If an additional current is flowing in parallel through the seal (Figure 1-17), it causes an increase in the standard deviations. For instance, if the current through the seal is ten-fold larger than through the channel, then the statistical fluctuations in current flow produced by the seal are $\sqrt{10}$ (316%) larger than they would be for a "perfect" seal.
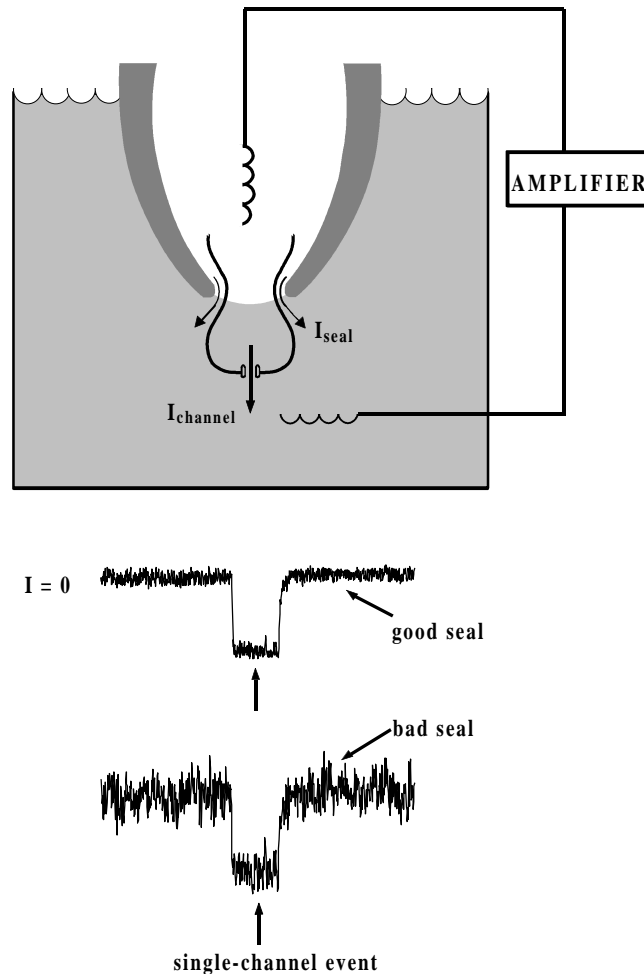
Figure 1-17. Good and Bad Seals

In a patch recording, currents through the seal also flow through the measuring circuit, increasing the noise on the measured current.

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