# Excitability in Plant Cells

*An external stimulus to a plant, such as touch, can trigger a cellular mechanism that generates a defensive response*

Randy Wayne

As a duck paddles along the edge of a pond, it nips at the tops of underwater vegetation. When one nip catches a shoot of *Chara*, a relative of the green algae, it sends a spectacular system into action. The force of the duck's bite triggers an electrical mechanism in the plant, and ionic current rushes across the membrane of the nibbled cell. Then the fluid inside the cell, the protoplasm, stops its normal flow around the periphery. The protoplasm quickly jells, preventing any leakage that could arise from the duck's attack.

*Chara* is hardly the only plant that responds to external stimuli. All plants respond to gravity as they grow, and plants can have various responses to light. Some follow a 24-hour cycle, adjusting the orientation of their leaves for the maximum absorption of light during the day. Some plants respond with movement when they are touched by predators.

What may be less obvious is *how* plants respond to stimuli. Although most people know that electrical signals mediate the responses of an animal's nervous system, it is less widely known that plant behavior, too, is governed by complex electrical mechanisms. Plant cells, in fact, are hotbeds of electrical activity, and plant studies have provided much of the foundation of what is known generally about electrical activity in cells. *Chara* has been important in those studies and continues to be.

Physiological studies of electrical activity began in the 19th century, and since then animal and plant physiologists have worked in parallel. In order to study the activity where it happens, at the cellular level, investigators had to find organisms in which the activity could be studied in isolation from the whole plant or animal. They also needed to find cells large enough that they could be probed with electrodes. In animal studies, the search led to the long nerve cells of squids, in which axons, the fibers carrying messages from the cell body, are so large that they were originally thought to be blood vessels. Plant physiologists, on the other hand, selected species of algae that have large cells, such as the characean algae *Chara* and *Nitella*.

In 1898 Georg Hörmann, a German physiologist, observed that big differences in voltage measurements could develop across cell membranes of *Nitella*. When such differences are regenerative they are called action potentials, because the regeneration implies action—the passing of an impulse. By the 1930s, characean algal cells were so well known that many investigators studied them. For example, K. S. Cole and Howard Curtis of the National Institutes of Health, who later became known as pioneers in the electrical excitability of squid neurons, began studying excitability in *Nitella*. These investigations showed that an action potential in *Nitella* is accompanied by a 200-fold increase in the cell membrane's conductance, as measured by the number of ions crossing the membrane. They concluded that ions carry the currents that create the action potential.

Although plants are no longer the leading organisms used in research on the basis of electrical excitability, a number of investigators have significantly advanced our knowledge of both the mechanisms and the effects of electricity in plants. Modern techniques common to neurophysiology have been applied to a variety of plants, and the results show that electrical physiology in plants is as complex as the systems found in animals. Moreover, a variety of plants use electricity to initiate action; examples are the closing of the leaves of a Venus flytrap and the touch-driven drooping of the leaves of some *Mimosa* species. Nevertheless, the most detailed information exists for characean algal cells, which I shall examine here. The electrical activity in these algae is worth examining not only for its importance in plant biology, but also because studies of plant excitability may help us understand the evolution of the human nervous system.

### Characteristics of Characeans

Characean algae have been used in much of the work on plant excitability. They are stoneworts, with a fossil record stretching back to the Devonian period, which began about 400 million years ago, and they are the ancestors of all higher plants. Extant stoneworts belong to a single family, Characeae, which is composed of six genera including *Chara* and *Nitella*. The majority of the extant species inhabit the bottom of clear freshwater ponds, where they live entirely submerged.

As I have noted, the primary attraction of characean algae as an object of study is the size of their cells. In *Chara*,

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Pat Lynch (Photo Researchers, Inc.)

**Figure 1.** *Chara*, an alga, responds to environmental stimuli, as do many plants. A variety of factors, including mechanical stimulation, can generate an action potential—a transient change in voltage across a cellular membrane—that causes some of this alga's internal fluid to jell, preventing it from leaking through small holes or tears in the plasma membrane. Large cells make *Chara* an appealing organism for electrical physiology. The plant's shoot is composed of long internodal cells separated by smaller nodal cells, seen here at the tip of a plant *(lower right)* and supporting reproductive structures *(top)*. Each internodal cell is about six centimeters long and half a millimeter wide; like all plant cells it has three distinct partitions. The outer surface is the cell wall, which is composed of cellulose. Underneath the cell wall is the plasma membrane, which is formed from two layers of lipids. Much of the inside of the cell is taken up by a vacuole, which is bound by the vacuolar membrane. (Photograph at right courtesy of the author.)

the plant body is composed of long internodal cells separated by smaller nodal cells. A single internode may be six centimeters long and half a millimeter wide, or about half as long as a toothpick and half as wide. The internal structure of an internodal cell is unlike that of an animal cell. Like all plant cells, the external border is a cell wall, which is composed of cellulose fibers that provide rigidity to the cell but are permeable to the extracellular fluid. Just beneath the cell wall is a semipermeable plasma membrane, which is composed of two layers of lipids that are interspersed with proteins. Beneath the plasma membrane there is a layer of chloroplasts, the sites of photosynthetic processes. Most of the interior of the cell is a vacuole, a sac filled largely with water and bounded by another membrane. The area between the vacuolar membrane and the plasma membrane is filled with protoplasm; here are found the cell nucleus and the cytoplasm, a viscous fluid that contains the cell's organelles such as mitochondria and ribosomes.

The protoplasm of characean cells moves constantly around the periphery

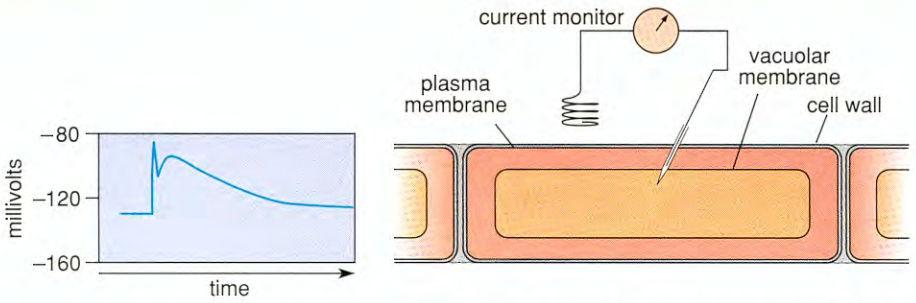


**Figure 2. Two components form an action potential in *Chara*. The voltages are recorded by inserting microcapillary electrodes into the cell, and the electrical output appears as a change in voltage across a membrane over time. With a reference electrode placed outside the cell and a microcapillary electrode inserted into the vacuole *(right)*, the two-component, whole-cell action potential is recorded *(left)* after stimulation. The action potential begins with a sharp spike followed by a more gentle hump. With the electrode arrangement shown above, the action potential makes the measurement less negative, or decreases the voltage across the cell. (Data from Shimmen and Nishikawa 1988.)**

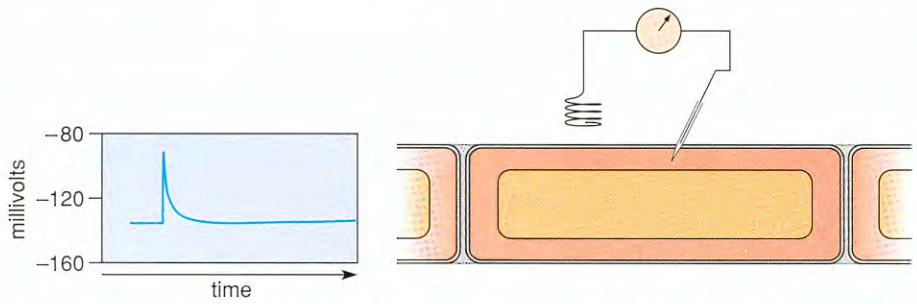


**Figure 3. Plasma membrane's action potential generates the sharp spike in an action potential of *Chara*. If a microcapillary electrode is inserted just through the plasma membrane *(right)*, the plasma membrane's action potential is recorded separately *(left)*. (Data from Shimmen and Nishikawa 1988.)**

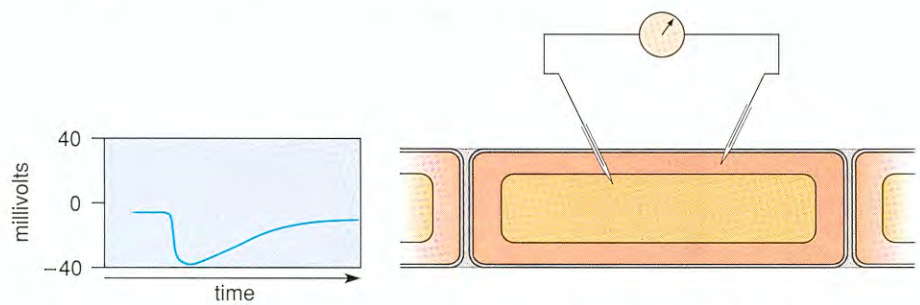


**Figure 4. Vacuolar membrane's action potential produces the gentle hump in an action potential of *Chara*. If one microcapillary electrode is inserted through the vacuolar membrane and a second microcapillary electrode is inserted through the plasma membrane *(right)*, the vacuolar membrane's action potential is recorded *(left)*. The recording of the vacuolar membrane becomes more negative, or hyperpolarizes, during its action potential because more negative charges accumulate in the protoplasm. (Data from Shimmen and Nishikawa 1988.)**

of the cell, just beneath the chloroplasts. The rotating belt of protoplasm travels at a speed of about 100 microns per second. Its movement, visible through a microscope, is called protoplasmic streaming or cyclosis. The streaming process is driven by the same interactions between actin and myosin that create contraction in muscles. The movement of the protoplasm mixes and transports molecules through the cell, which would take too long in such large cells if diffusion were the only mechanism available.

A fundamental concept in electrical physiology is defined by the term *potential*. A potential is a voltage across a membrane, which is created by the separation of positive charges from negative charges. In biology, charges are carried by ions. Positive charges are carried by cations such as sodium, and negative charges are carried by anions such as chloride. If one side of a membrane has more positively charged ions and the other side has more negatively charged ions, then there is a potential, or voltage, across the membrane.

Here I shall discuss four potentials: membrane potential, resting potential, receptor potential and action potential. A membrane potential is the voltage across a membrane, or a measurement of the distribution of ions. The resting potential is the membrane potential when the cell is not being stimulated. Both a receptor potential and an action potential change the membrane potential. A receptor potential arises when a receptor in a membrane, such as a molecular mechanoreceptor, is stimulated. The stimulation generates an ionic current that changes the membrane potential, but the receptor potential decreases in magnitude with distance from the stimulated receptor. An action potential is a large, transient change in the membrane potential that is self-perpetuating, or regenerative, and it can travel the length of the cell without decreasing in magnitude.

Characean algae generate action potentials when subjected to a variety of stimuli, including a sudden change in temperature, ultraviolet radiation, odorants and mechanical action. These stimuli first cause the plant to produce a receptor potential. For example, a small mechanical stimulus is converted by a receptor into electrical energy that is proportional to the magnitude of the stimulus. In a resting characean cell, there is a negative voltage inside the plasma membrane relative to the outside of the cell. In other words, there are more negatively charged ions inside the membrane and more positively charged ions outside the membrane. The receptor potential generates depolarization, a decrease in the voltage difference between the inside and the outside of the cell. This potential generally lasts as long as the stimulus is present, and it is essentially an electrical replica of the stimulus. If the stimulus depolarizes the cell to a specific threshold level, an action potential is generated.

An action potential in one area of a characean cell causes protoplasmic streaming to stop throughout the cell. As I shall explain below, the action potential causes external calcium to move into the protoplasm. The increased calcium concentration activates a protein kinase that adds a phosphorus group to myosin and thereby inhibits its interaction with actin, which stops the driving force behind protoplasmic streaming.

The cell may also become isolated from neighboring cells because the streaming usually enhances the passage of substances between cells via small tubes known as plasmodesmata. When the action potential abates, calcium is pumped from the protoplasm, and streaming resumes.

**Probing the Potential**

It is generally an intricate task to record the precise electrical activity of any cell. Such measurements are best made intracellularly—recording the voltage difference between the outside and the inside of the cell. This is done by placing a reference electrode outside the cell and a recording electrode inside the cell. In many neurons, such recording requires the use of an air table to isolate the preparation from the slightest movement, a microscope with magnification of as much as 250 times, and a micromanipulator, a mechanical device that controls small, precise movements of the electrode. In *Chara*, however, intracellular recording is easy; it is even possible to do it with a naked eye guiding the movements and a relatively steady hand holding the electrode, although investigators employ a low-magnification microscope and a micromanipulator to further simplify the task.

The characean action potential moves away from the receptor in both directions along the cell at a speed of 0.01 to 0.4 meters per second. This is much slower than the so-called conduction velocity of action potentials in nerves, which is between 0.4 and 42 meters per second depending on the specific nerve and organism. When an animal's muscle is stimulated, it produces an event that has been called E-C coupling, or excitation-contraction coupling, because the electrical excitation causes the muscle to contract. In characean cells, electrical stimulation produces a different kind of E-C coupling, excitation-cessation coupling. In algae this refers to the fact that electrical stimulation causes the cessation of protoplasmic streaming.

When an electrode is inserted into a characean vacuole and a reference electrode is placed outside the cell, an action potential can be observed after stimulation *(Figure 2)*. The response appears to contain two components: a fast component and a slow component. In fact, it is two separate action potentials. The fast potential is across the plasma membrane *(Figure 3)*, and the slow one is across the vacuolar membrane *(Figure 4)*.

With no external stimuli, the voltage difference across a cellular membrane is called the resting potential. In characean cells, the average resting potential is –180 millivolts across the plasma membrane and –10 millivolts across the vacuolar membrane. (The negative sign indicates that the protoplasmic side is negative with respect to the other side of the membrane. That is, the plasma membrane is negative on the inside and positive on the outside, and the vacuolar membrane is negative on the outside and positive on the inside.) During an action potential, the plasma membrane depolarizes to about zero millivolts, making the inside and the outside of the cell about equal in charge; the vacuolar membrane hyperpolarizes (meaning that it becomes more negative) to about –50 millivolts.

The changes in membrane potential that develop during an action potential arise from ionic currents that flow as a consequence of a change in a membrane's permeability to specific ions. The changes in permeability that develop can be measured as the specific conductance of the membrane. This is a measurement of the membrane's permeability to all ions; it is given in a unit called a siemens (the reciprocal of resistance or $ohm^{-1}$, sometimes called a mho) per square meter. At rest, the specific conductance is 0.83 siemens per square meter for the plasma membrane and 9.1 siemens per square meter for the vacuolar membrane. The specific conductance changes during the action potential, and the peak specific conductance is 30 siemens per square meter for the plasma membrane and 15 siemens per square meter for the vacuolar membrane. This result reveals that an increase in ionic conductance accompanies an action potential, but it does not indicate which ions are crossing the membranes and carrying the currents that create an action potential.

**Particular Permeabilities**

The electrical potential across a membrane is largely determined by the differences in ionic concentrations on the inside and the outside of the membrane. The ions of interest in most organisms are calcium, chloride, sodium and potassium. Since characean cells include two membranes, there are three fluids of interest: the extracellular fluid (the fluid outside the cell), the protoplasm (the fluid between the plasma

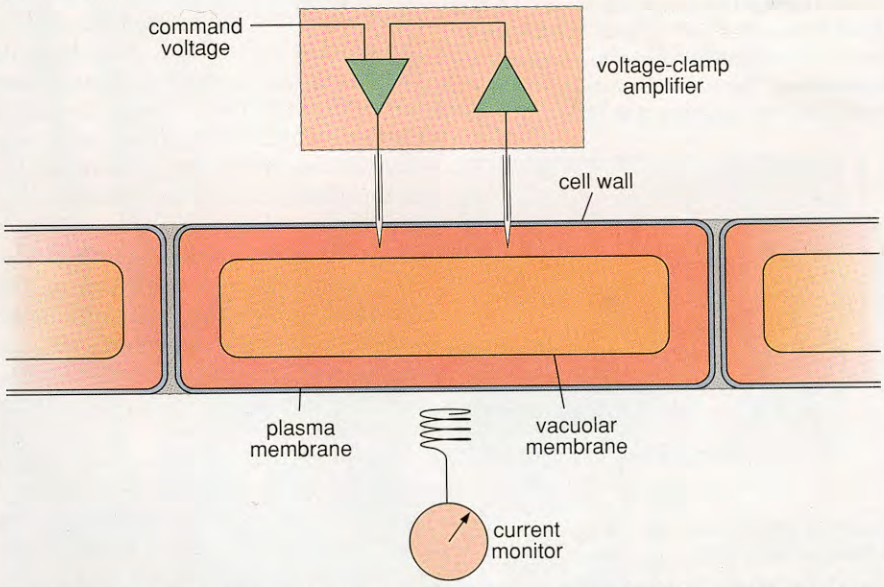


**Figure 5. Voltage clamping reveals ionic currents that move across a membrane. A voltage clamp holds, or clamps, the voltage across a membrane at a value called the command voltage. An electrical circuit compares the command voltage with the actual membrane potential and injects the appropriate current to minimize the difference. The current passed by a voltage clamp is measured, and it is effectively a mirror image of the ionic current flowing across the cell's membrane. When the membrane is clamped at the resting potential, no current is passed by the voltage clamp because the membrane is in equilibrium. If the membrane is clamped at voltages other than the resting potential, the voltage clamp passes current to offset the current that flows across the cell's membrane. Here the plasma membrane is clamped by two microcapillary electrodes. The electrode on the right records the voltage across the plasma membrane. A voltage-clamp amplifier compares the plasma-membrane voltage to the command voltage and then injects current through the microcapillary electrode on the left. An extracellular electrode monitors the current flowing across the membrane.**