Excitability in Plant Cells

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

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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,
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.

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.
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 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 micro-manipulator, 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. 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 about -180 millivolts across the plasma membrane and about -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...
membrane and the vacuolar membrane) and the vacuolar fluid (the fluid inside the vacuole). The concentrations of ions can be given in the ratio of extracellular concentration to protoplasmic concentration to vacuolar concentration, because only the relative values are significant to this discussion. The average ionic-concentration ratios are 100:1:12,000 for calcium, 1:55:405 for chloride, 1:50:340 for sodium and 1:1,100:1,030 for potassium. In other words, the concentration of calcium is low in the protoplasm and high in the vacuole; the chloride concentration is higher in the protoplasm than in the extracellular fluid, and higher still in the vacuole; the distribution of sodium is similar to that of chloride; and potassium has a higher concentration in both the protoplasm and the vacuole.

The location of an ion is determined by a chemical force and an electrical force (Figure 8). In response to the chemical force, an ion tends to go from an area of higher concentration to an area of lower concentration. The electrical force pulls an ion toward an area of opposite charge, so that a cation, or positively charged ion, is drawn toward a negative area. Consider a potassium ion in the protoplasm. Potassium is more concentrated in the protoplasm than in the extracellular fluid, and thus the chemical force tends to pull potassium out of the cell. The resting potential of the plasma membrane, however, is negative in the protoplasm relative to the extracellular fluid. This creates an electrical force that pulls potassium, a cation, from the extracellular fluid into the protoplasm. At equilibrium, the chemical and electrical forces balance, and there is no net movement of ions, or charge. Therefore, an uneven distribution of ions can create a stable membrane potential.

The membrane acts like a capacitor, a component that separates electrical charge. By knowing the difference in an ion’s concentration across a membrane, it is possible to calculate the voltage difference, or potential, at which the chemical and electrical forces will be balanced for that ion. This potential is called the equilibrium potential or the Nernst potential. For potassium, the German physical chemist who derived it, the equation follows:

$$E = \frac{RT}{zF} \ln \left( \frac{C_0}{C} \right)$$

In this equation, E is the Nernst potential, R is the universal gas constant (8.31 joules per mole per degree in Kelvin), T is temperature in degrees Kelvin, z is the ion’s valence, F is the Faraday constant (9.65 x 10^4 Coulombs per mole), C_0 is the ion’s concentration on the outside of the membrane and C is the ion’s concentration on the inside of the membrane. By assuming a temperature of 20 degrees Celsius or 293 degrees Kelvin, which is approximately room temperature, the equation can be simplified to:

$$E = \left( \frac{58}{z} \right) \log \left( \frac{C_0}{C} \right)$$

This equation gives E in millivolts. Considering the Nernst potential for sodium across the plasma membrane, the equation would be:

$$E = \left( \frac{58}{z} \right) \log \left( \frac{1}{50} \right) = -98.5$$

(For sodium, z = 1.) This means that sodium would be in equilibrium across the plasma membrane at a potential of -98.5 millivolts.

Each ion has a Nernst potential. In characean cells, the average Nernst potentials for the major ions across the plasma membrane are 59 millivolts for calcium, 103 millivolts for chloride, -100 millivolts for sodium and -180 millivolts for potassium.

The resting membrane potential arises from the combined equilibrium potentials of all of the ions. You may have noticed, however, that both the resting potential of the characean plasma membrane and the Nernst potential for potassium are -180 millivolts. This is not merely a coincidence. It has been shown that in resting characean cells, as well as in most resting animal nerves, the membrane is largely impermeable to calcium, chloride and sodium, but it is readily permeable to potassium. This means that the resting potential is largely determined by the passive diffusion of potassium. During an action potential, the membrane’s permeability to specific ions changes.

**Ions in Action**

Ionic movement generates action potentials in animal, plant and fungal cells. In 1949 Alan Hodgkin and Bernard Katz, both then at Cambridge University, showed that external sodium is necessary for an action potential in a squid nerve. Through a series of experiments, they developed the sodium hypothesis, which states that the massive depolarization of an action potential results from sodium rushing into a cell. It was later shown that tetrodotoxin (the deadly poison found in the Japanese puffer fish and removed before the fish is eaten as sashimi) prevents an ac-
Membrane potential, or voltage, depends on the relative strength of a chemical force and an electrical force. This can be seen in the movement of potassium ions (K⁺) across the plasma membrane of Chara. Potassium ions are about 1,000 times more concentrated in the protoplasm inside the membrane than in the extracellular fluid outside. The ions tend to move from areas of higher concentration to areas of lower concentration (left). This chemical force drives potassium ions out of the protoplasm into the extracellular fluid. The movement of positive ions out of the cell causes the plasma membrane to have a positive charge on its extracellular side and a negative charge on its protoplasmic side. Since opposite charges attract, an electrical force pulls positively charged potassium ions from the extracellular fluid into the protoplasm (right). At a membrane voltage called the equilibrium potential or the Nernst potential, the chemical force and the electrical force balance so that there is no net movement of potassium ions.

The Nernst potential for sodium in characean algae is not large enough to create the large depolarization that develops at the plasma membrane during an action potential. The Nernst potential for sodium drives it into the cell, but once the cell depolarizes from its resting potential of −180 millivolts to sodium’s Nernst potential of about −100 millivolts, sodium no longer moves into the cell. Nevertheless, a characean action potential depolarizes the plasma membrane to about zero millivolts. This suggests that calcium or chloride contributes to the depolarization. Calcium (a cation with two positive charges) could depolarize the cell by moving in, and chloride (an anion with one negative charge) could depolarize the cell by moving out. Potassium is not a candidate because the depolarization during an action potential moves away from potassium’s Nernst potential.

Disagreement arose about the importance of calcium and chloride. In the early 1960s Geoff Findlay and Alex Hope of the University of Sydney in Australia showed that the magnitude of depolarization depends on the concentration of the external calcium, and they believed that calcium carries the current of the action potential. Nevertheless, when they employed radioactive calcium as a tracer, they were unable to detect any calcium moving into the cell. At the same time, Lorin Mullins of the University of Maryland proposed that calcium simply activates a mechanism that causes chloride to move out of the cell. It was shown that the level of chloride leaving the cell increases 100 fold during an action potential. This amount of chloride moving...
The above work and other studies suggested that it is the exodus of chloride from the cell that is responsible for the massive depolarization. After the action potential ends, the plasma membrane returns to its resting potential because potassium moves out of the cell, again making the inside more negative than the outside.

Clamping and Currents

In the above experiments, a current was applied to a cell and then the change in the membrane potential was measured. Another way of measuring electrical activity in cells uses the voltage clamp, which was developed by K. S. Cole in 1949. A voltage clamp holds, or clamps, the membrane potential at a set value (Figure 5). This is accomplished through an electrical circuit that continually compares the desired clamp potential with the actual membrane potential, and injects the appropriate current to minimize the difference. The current passed by a voltage clamp can be 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 a negative potential, less current flows into the cell. The current then decreases because the channel closes.

The quick, transient current lasts for several hundred milliseconds. When the plasma membrane is depolarized to ~50 millivolts this current flows inward. At more depolarized potentials, the current moves outward. The reversal potential for the quick, transient current is between ~60 and ~20 millivolts. Studies have shown that this current arises from calcium ions moving across the plasma membrane. The reversal potential of this channel, however, is not equal to the Nernst potential for calcium because the channel is permeable to a number of cations.

The slow, transient current appears when the plasma membrane is depolarized to between ~90 and ~120 millivolts. Its reversal potential depends on the external concentration of chloride ions. If the external chloride concentration is decreased, the reversal potential of the slow, transient current changes according to the Nernst potential for chloride.

In addition, the slow, transient current can be blocked with ethacrynic acid and anthracene-9-carboxylic acid—well-known chloride-current blockers. The slow, transient current, then, is a chloride current.

These voltage-clamp experiments confirm that the depolarization of the plasma membrane develops as a result of the movement of calcium into the cell (the quick, transient current) and chloride out of the cell (the slow, transient current). After these two currents stop, the steady-state current returns the plasma membrane to its resting potential through the flow of potassium out of the cell. Figure 7 shows the temporal relationship between these ionic flows.

Lunevsky and his colleagues postulated that a chloride channel, which is specific for the permeability of this ion, is activated by an increase in the protoplasmic concentration of calcium, which arises from the quick, transient current. The calcium channel is initially activated by the receptor potential that is generated by a stimulus. If the calcium channel is blocked, the chloride current does not appear, suggesting a causal relationship between the two currents.

Checking Out a Channel

A current flows through channels that are composed of protein molecules embedded in the membrane. These molecules create little pores through which ions pass. The current through a single channel can be recorded with the patch-clamp technique. In this technique, a glass microcapillary electrode is placed against the surface of the membrane. Suction is applied through the electrode, and a tiny piece of the plasma membrane (about one square micron) is pulled away, sealed to the electrode like the head on a drum. If the patch is
small enough, and in the right place, it may contain a single channel. The isolated membrane patch does not have a natural membrane potential, but a voltage clamp can be used to set the potential to any value. Typically, a channel opens at a specific potential, and the flow of ions can be measured as current, usually in the range of picoamperes, or trillionths of an ampere. When the channel is closed, no current flows.

Kiyoshi Okihara and his colleagues at Osaka University applied patch-clamp techniques to the plasma membrane of a characean cell, and they identified the calcium-activated chloride channel responsible for the plasma membrane's action potential. Current passes through this channel only when the calcium concentration in the protoplasm is about 1 micromolar, approximately 10 times its normal concentration. If the protoplasmic calcium concentration is higher or

Figure 11. Characean action potential arises from a cascade of processes. At rest, both the plasma membrane and the vacuolar membrane are in electrical equilibrium, and protoplasm streams through the space between the two membranes. An external stimulus, such as touch, generates a depolarization across the plasma membrane called a receptor potential. No one knows which ion causes the receptor potential. The receptor potential causes calcium ions to move from the extracellular fluid into the protoplasm (a). Some of the calcium ions activate chloride channels in the plasma membrane, allowing chloride ions to move from the protoplasm to the extracellular fluid (b). This outward chloride current depolarizes the membrane, which is negative on the inside at rest, and the current generates an action potential across the plasma membrane. The action potential moves along the cell because the depolarized membrane opens more calcium channels, which open more chloride channels. Calcium ions continue diffusing through the protoplasm, and some of them open calcium-activated chloride channels on the vacuolar membrane, allowing chloride ions to move from the vacuolar fluid to the protoplasm (c). This chloride current generates a hyperpolarization of the vacuolar membrane because chloride ions have a negative charge and the vacuolar membrane is already negative on the protoplasmic side. The calcium ions in the protoplasm stop protoplasmic streaming by inhibiting the actin-myosin system that drives the streaming. At about the same time, potassium ions flow from the protoplasm to the extracellular fluid, which stops the plasma membrane's action potential by returning the membrane to its polarized state. Finally, potassium ions flow from the vacuolar fluid to the protoplasm, which stops the vacuolar membrane's action potential, and calcium ions are pumped from the protoplasm, allowing streaming to resume (d).
lower than normal, the current decreases. The channel apparently needs calcium to open, but too much calcium causes it to close. No one knows how a high calcium concentration inhibits the channel.

The reversal potential of the calcium-activated chloride current depends on the chloride concentration as predicted by the Nernst potential for this ion. Moreover, the channel is voltage-dependent. In other words, the channel will open only at specific plasma-membrane potentials. In a simple form, a voltage-dependent channel could be created from a membrane-bound molecule with a large dipole moment, one end positive and the other negative. The positive end would swing toward the negative side of the membrane, and this movement could place the molecule across an opening in the membrane, closing the channel. If the potential across the membrane were to switch, the molecule would swing around, which might uncover or open the channel. The calcium-activated chloride channel opens when the membrane is depolarized to potentials less than -160 millivolts. This reveals that a membrane depolarization, such as a receptor potential, is necessary to open the calcium-activated chloride channel, but depolarization is not enough. Current will flow through the channel only if the membrane is sufficiently depolarized and if both calcium and chloride are present.

Inside Action
As I mentioned earlier, a characean cell has three compartments that bathe two excitable membranes: the plasma membrane and the vacuolar membrane. An animal cell has only one membrane, and this makes an axon much simpler than a characean cell. An axon is geometrically similar to an electrical cable, and thus cable theory can be easily applied to such a system. This is the reason that Cole and Curtis began studying the squid axon rather than continuing with characean cells. The vacuolar membrane of a characean cell adds a second component to the action potential.

At rest, the vacuolar membrane potential is about -10 millivolts. In this case, the membrane is negative outside the vacuole, which is the reverse of the plasma membrane. During an action potential, the vacuolar membrane hyperpolarizes (becomes more negative outside the vacuole) to about -50 millivolts. The average Nernst potentials at the vacuolar membrane are 121 millivolts for calcium, -51 millivolts for chloride, 49 millivolts for sodium and -2 millivolts for potassium. These numbers indicate that chloride is the only ion capable of carrying the vacuolar membrane potential to -50 millivolts. Chloride moves out of the vacuole to make the membrane potential more negative.

In a characean cell, the plasma membrane and the vacuolar membrane interact. The vacuolar membrane, however, can be studied separately by making the plasma membrane permeable to all ions—essentially, by making it disappear. This is done by placing a cell in a calcium-free solution that contains EGTA (ethyleneglycol-bis-tetraacetic acid)—a calcium chelator—and is at 4 degrees Celsius. This treatment stops protoplasmic streaming because ATP escapes from the cell. Streaming can be reactivated by adding ATP to the bathing solution.

In a so-called plasma-membrane-permeabilized cell, a vacuolar-membrane action potential can be initiated by increasing the calcium concentration of the bathing solution from zero to one micromolar. If the chloride concentration is increased in the protoplasm, the calcium-induced action potential decreases as predicted by the Nernst equation, if chloride is assumed to be the only current-carrying ion. Anthracene-9-carboxylic acid, a chloride-channel blocker, completely eliminates the potential. Minehiro Kikuyama of the University of the Air in Japan measured the movement of chloride from the vacuole to the protoplasm and found that an increase in protoplasmic calcium creates an increase in chloride moving out of the vacuole. If the fluid in the vacuole is replaced with a chloride-free solution, there is no increase in protoplasmic chloride, indicating that the chloride does come from the vacuole.

In intact cells, the vacuolar mem-
brane's action potential develops only after the plasma membrane has been excited. This indicates that some mechanism couples the plasma membrane's action potential to the vacuolar membrane's action potential. Calcium is assumed to be the coupling agent for several reasons. First, a characean action potential increases the concentration of calcium in the protoplasm. Second, removing calcium from a cell's external solution does not affect the plasma membrane's action potential (as long as the calcium is replaced with a similar ion, such as barium), but this does inhibit the vacuolar membrane's action potential. And finally, a microinjection of calcium into the protoplasm generates an action potential in the vacuolar membrane.

It is now possible to describe the fundamental steps in a characean action potential (Figure 11). An external stimulus causes a depolarization of the plasma membrane (a receptor potential). The receptor potential arises from the movement of calcium through the plasma membrane and into the protoplasm. If enough calcium moves into the protoplasm, it depolarizes the plasma membrane enough to open the calcium-activated chloride channels, generating additional depolarization as chloride passes out through the plasma membrane. The calcium diffuses across the five to 20 microns of protoplasm to the vacuolar membrane at a speed of about one micron per second. At the vacuolar membrane, the calcium activates chloride channels on the membrane, allowing chloride to move from the vacuole into the protoplasm, which hyperpolarizes the second membrane. The plasma membrane's action potential ends as potassium leaves the protoplasm, and the vacuolar membrane's action potential ends as potassium moves from the vacuole to the protoplasm.

Other Excitable Plants
Other plants employ electrical signals to elicit behaviors and physiological processes. Although the mechanistic explanations for many plant responses have only recently emerged, the responses themselves have been known for some time. Charles Darwin, among others, noted that many plants respond to mechanical stimulation. Darwin became interested in carnivorous plants such as the Venus flytrap, which he called “one of the most wonderful in the world,” and he was the first to show that the plant digests captured insects.

At rest, the lobes of a Venus flytrap sit passively open. Each lobe secretes a type of nectar that attracts insects, and so-called trigger hairs are embedded in the inner surface of each lobe. If an insect steps on a lobe and either hits two trigger hairs or hits the same hair twice, the mechanical stimulation generates an action potential, and the lobes close, capturing the insect.

The sundew gets its name from its appearance. This plant shines as if coated with dewdrops because it is covered with sticky hairs that can capture insects. Once an insect is captured, it begins to struggle, and the mechanical stimulation to the plant induces action potentials that cause the hair to wrap around the insect. Neighboring hair cells also produce action potentials, and they, too, wrap around the insect, thus

Figure 13. Sundew entangles insects in the plant's sticky hairs, which adhere to an insect's feet. When the insect tries to escape, the mechanical stimulus to the plant generates an action potential that causes the hair to wrap around the insect. Neighboring hairs also produce action potentials, and these hairs, too, wrap around the insect. Secretory cells then release enzymes that digest the insect.
providing a secure trap. Then nearby secretory cells exude enzymes, forming a little stomach that digests the insect.

One of the best-known examples of plant behavior comes from *Mimosa pudica*, often called the sensitive plant. When the leaves of the plant are touched, they bend over and appear dead. The drooping arises from a mechanically driven action potential. Moreover, an action potential propagates from the stimulated region throughout the plant. This causes drooping in the rest of the plant, a defense mechanism apparently designed to make the whole plant look unappealing.

Not all plant action potentials, however, cause obvious responses. In *Luffa*—the plant whose gourd or fruit is used for "loofah" sponges—action potentials cause a transient inhibition of growth. And in a variety of flowers, pollen landing on the stigma generates an action potential, which may be involved in subsequent pollination or the maturation process. In tomato seedlings, a mechanical wound induces electrical activity that causes the accumulation of proteins that limit further damage to the plant.

Electrical phenomena control many responses in plants. In a characean alga, we understand many of the details of the mechanism that leads from a duck's nip on the plant to the cessation of protoplasmic streaming. But we are just beginning to address the similarities between the electrical excitability in characean algae and higher plants, let alone animals. In any case, it is apparent that plants can perform long-distance communication through electrical signals, such as the passing of information from a mechanical stimulus from one *Mimosa* stem to another. Many biologists continue to describe electrical excitability as part of the animal world. In the future, we should think of plants as excitable too.

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