Patch clamping and its use in the study of photoreceptors

Cellular Bioengineering, 2006

The plasma membrane is highly important to cell life and activities. One of its many functions is the formation of ion channels, which are proteins that span the membrane and create a pore through it. The channels create voltage gradients across the membrane by the selective accumulation and flow of solutes. These gradients play a role in cell growth and development, nutrition and responses to external signals, among other processes. The field of electrophysiology, which is the study of charged solute transportation across membranes [1], has shed light on the mechanisms involved in cell transport at the molecular level. One technique used extensively in this field is patch clamping. Patch clamping involves pressing the tip of a glass pipette against a cell membrane and forming an electric seal, thereby allowing the measurement of current and voltage changes across the membrane through ion channels [1]. The details of this procedure as well as a specific use of the technology involving photoreceptors will be discussed.

Introduction to patch clamping

A major step that must be completed before patch clamping can be performed is forming the pipette tip that will ensure a tight, high-resistance seal against the cell membrane. To do this, a glass capillary tube is heated and then pulled apart (Figure 1). As it is pulled, the diameter at the middle thins to on the order of 10 μm, leaving a steeply tapered point [2]. Changes in the amount of heat used during this process can precisely vary the size of the opening down to fractions of a micrometer [1]. Once the tip of the pipette is heat polished to make it smooth and flat, it is ready to use in a patch-clamping experiment.

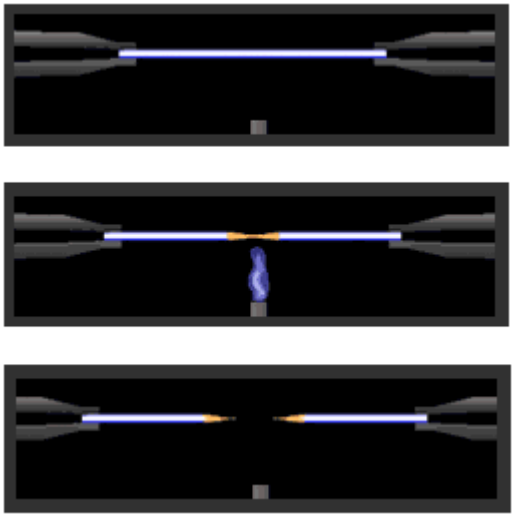

Figure 1: The steps involved in making a pipette for patch clamping include heating a capillary tube and pulling it apart [http://www.cellsalive.com/patch.htm].

The pipette, once connected to a system of rubber tubing, an electrode, and a signal amplifier, is filled with buffer and held in a stage that can be precisely manipulated in the x, y and z planes (Figure 2). With the aid of a light microscope, the pipette is brought near to a single cell in a dish of cells that are bathed in a buffered solution. Once contact is made with the cell membrane, suction is applied to the end of the rubber tubing, usually by mouth. By watching the cell through the microscope, the formation of a seal between the pipette tip and the plasma membrane can be seen. The seal remains intact when the suction is halted, and is termed a gigaseal [2]. The resistance between the pipette tip and the surface of the cell membrane is normally between 50 and 200 MΩ [3]; the high resistance guarantees that the currents originating in that particular section of membrane at the pipette opening will flow directly into the pipette and can then be measured.

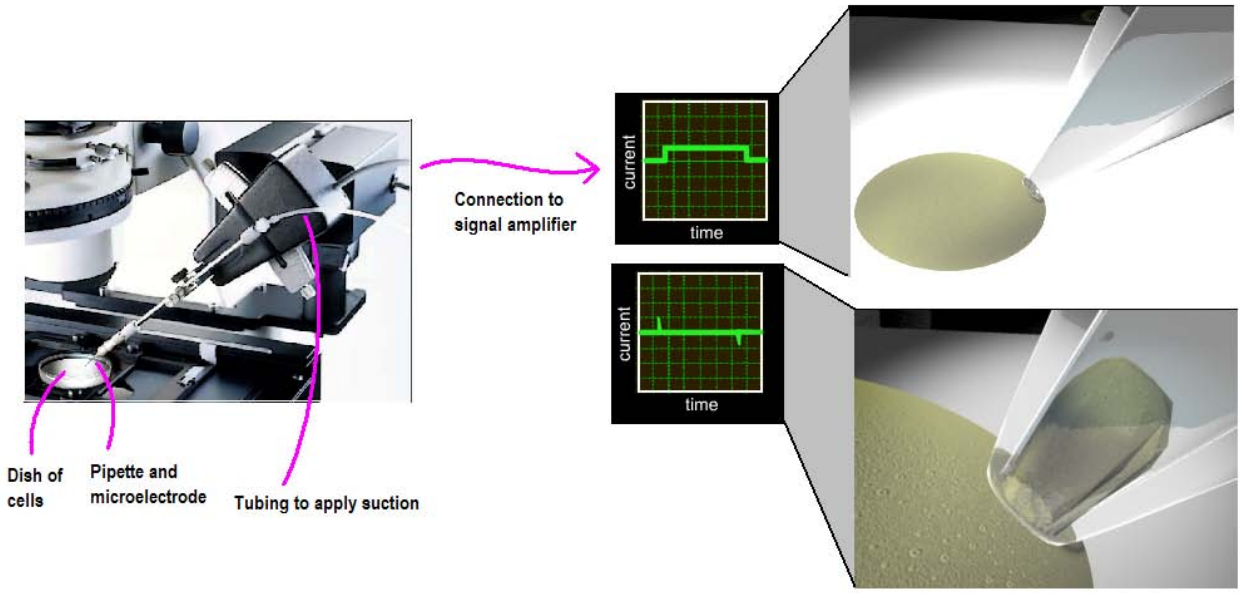

Figure 2: The setup used to record patch clamping data includes a dish of cells into which a pipette and an electrode are placed. These are connected to rubber tubing and an amplifier, which displays the current across the membrane versus time. The pictures at the right show the typical signals seen when the pipette comes in contact with the cell and suction is applied [http://www.science-display.com/patchclamp.html].

There are several configurations for recording patch clamping data (Figure 3). The currents derived from ion flux through a single ion channel can be measured if the membrane patch is removed from the cell. This is accomplished by forming a seal and then retracting the pipette from the cell, leaving the patch of membrane on the tip of the pipette. This is known as the inside-out patch. Alternatively, a voltage is applied via a circuit with a power source that introduces charge directly into the pipette before the pipette is pulled away from the cell. This results in the rupturing of the cell membrane, causing the pipette solution to equilibrate with the cell cytosol. Once the pipette is moved away from the cell, an outside-out patch is

formed. The final configuration, which the remainder of this paper will focus on, is whole-cell mode. The formation of this setup is identical to outside-out mode, the difference being that the pipette is never released from the cell [4]. This is used for recording currents across the entire plasma membrane of the cell, rather than a single channel [4].

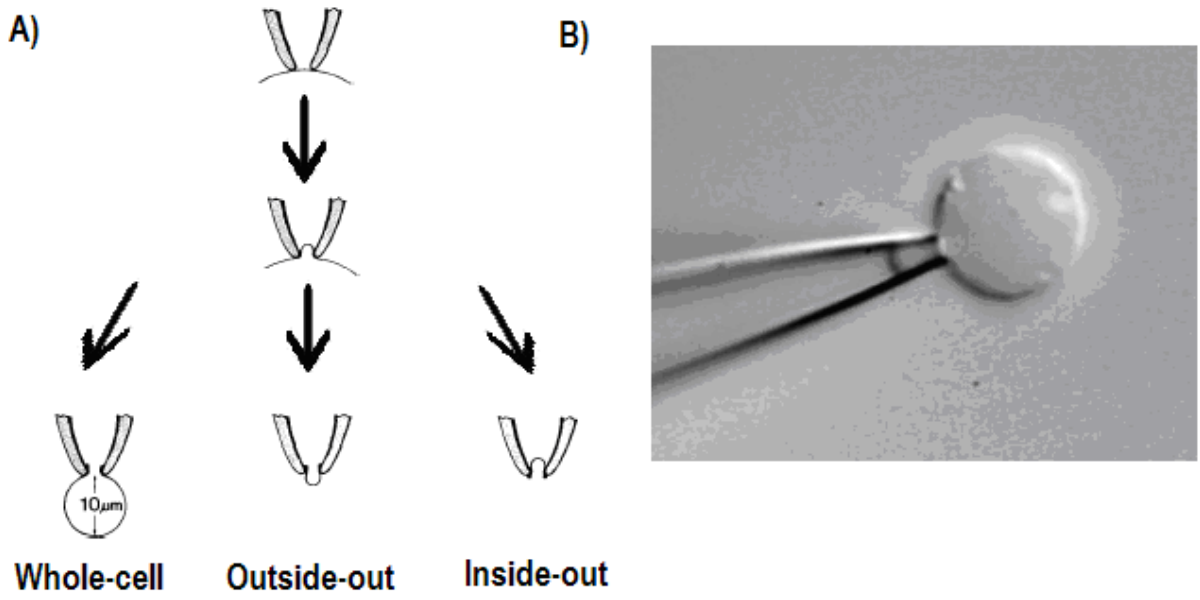

Figure 3: A) Whole-cell mode is obtained by applying suction to the pipette when it is touching the cell membrane and then leaving the cell intact. If the pipette is pulled away from the cell, outside-out mode results. Inside-out mode results from pulling the pipette away from the cell membrane before the membrane is broken by suction. B) A patch of cell membrane moves into the tip of the pipette when suction is applied.

Once the pipette solution is in equilibrium with the cell cytoplasm, data can be collected by what is known as probing the cell. This involves manipulating one variable that plays a role in ion channel function and measuring its effects on another factor. The current or voltage is recorded using a microelectrode that is inserted into the cell via the pipette. A reference electrode is placed in the extracellular fluid and is connected to a potentiometer that, along with an amplifier, provides a readout of potential differences by converting the ion currents into electrical currents. The measured potential can be as small as a few picoamps to as large as several hundred picoamps [1].

The most common way to collect such data is with the use of a voltage clamp. In this case, the membrane potential is controlled by the experimenter while the current is measured [3]. The ion channels in the membrane are responsible for the currents generated, and their activity depends on the potential differences between the inside of the cell and the outside environment. As an example, neurons send

action potentials through their axons only if a certain threshold potential is reached by the flux of sodium and potassium ions through the membrane. The voltage of an inactive cell remains at a negative value and, when depolarized, the cell will undergo an action potential. A whole-cell patch clamp on a neuron would allow the researcher to control the voltage difference between the inside and outside of the membrane. These changes, analogous to the natural potentials that develop in neural cells, will often lead to activity of ions through membrane channels. This causes the cell to induce currents which can be measured and recorded [3].

Similarly, a current clamp can be used to collect data. In this case, the current is controlled while the changes in membrane voltage are recorded [3]. Going back to the neuron example, currents through the cell's sodium and potassium ion channels determine the voltage differences that will result in the transfer of an action potential. These currents can be controlled by the experimenter by changing the solution in the pipette or in the extracellular environment. Since the pipette solution equilibrates with the inside of the cell, voltage differences can be generated by the cell membrane in response to these changes. For example, if a solution rich is Na^+ ions is introduced to the pipette, the neuron will respond by opening K^+ ion channels in order to return to a resting membrane potential. In most cases, voltage clamp and current clamp modes are used in tandem in order to evaluate the cell setup as completely as possible [2].

The major weak point of patch clamping is the extensive practice required in order to obtain valuable data. It is quite difficult to approach the cell with the pipette and form a seal without damaging the cell. Moving the pipette even the slightest bit too far when advancing toward the cell can rupture the cell or cause the pipette to hit the bottom of the dish and break. When a mishap occurs, the dish of cells or the pipette (sometimes both) must be replaced – a time consuming process. A researcher with the skills to get the pipette tip to the cell without damage has other concerns, though. Applying suction with too much force or for too long can also harm the cell, sometimes causing the entire cell to be sucked into the pipette. In addition to closely monitoring the location of the pipette in relation to the cell, the level and quantity of cell bath solution and the oscilloscope must be watched closely [1]. One finds that an extra set of eyes or hands would be beneficial.

Aside from the patience required for patch clamping, most limitations of the whole-cell technique have diminished in the past few decades due to more precise recording devices and methods for reducing noise in the system [4]. The resolution of a patch clamp is limited by the noise generated by the amplifier (around

0.2 pA); however, processing the data after collection can often remove the effects of noise. Fortunately, currents generated by just one ion channel are often greater than 1 pA, making resolution an issue only in some cases.

Another limitation is the large size of the pipette interior compared to the small size of the cell. When the cell interior is replaced by the fluid in the pipette, some important cellular components are taken out of the system. This can potentially lead to alterations in cell behavior, making it difficult to determine the exact mechanisms of cellular functions. Because of this, data can be taken from a cell for about 10 minutes before it must be replaced with a new cell [2]. If the extracellular fluid has been manipulated, then it is necessary to replace of the entire dish of cells before recording can continue.

Patch clamping in the study of photoreceptors

Whole-cell patch clamping has been used in biology and bioengineering research in several cell types for a variety of purposes. The ion fluxes in a neuron were used above as a general example to explain the technique; however, in order to fully comprehend the advantages of measuring current differences across a cell membrane, a specific example will be helpful. One use of patch-clamping recently has been the study of action potentials in a particular type of neuron - the photoreceptor.

Photoreceptors are found in the retina and send signals, like other neurons, by changes in membrane potential. A light stimulus hyperpolarizes a photoreceptor, causing a flux of ions through the cell membrane. The potentials that arise allow for differentiation of color and intensity. Up until the past few years, it has been unclear which types of ion channels are involved in photoreceptor function. Kawai *et al.* used the whole-cell patch clamp technique with pieces of human retina to measure currents and voltages through the membrane when potassium ions were introduced to the inside of the cell through the pipette [5]. To ensure that the photoreceptors were not damaged during the process, they were kept intact with the retinal tissue and differentiated from other cell types by the use of staining [5].

To test if the photoreceptors could generate action potentials, they recorded the voltage changes when a depolarizing current was introduced to the cell. The current, around +60 pA, induced an action potential. They then added tetrodotoxin (TTX), a neurotoxin known to block voltage-gated sodium channels [6], to the pipette solution and observed that the action potential was blocked. The voltage was then clamped at -100 mV, and they observed a steady inward current of 250 pA [5]. Once again, they introduced TTX to the

system and the action potential was blocked. They were able to conclude that action potentials in human photoreceptors are from Na^+ (Figure 4a). Then, by changing the values of the currents and voltages they applied to the cell, they determined specific values necessary to evoke a cellular response [5].

Contrary to the action potentials seen in human photoreceptors, cold-blooded vertebrates do not express voltage-gated Na^+ channels. Instead, their photoreceptors use voltage-gated calcium channels to generate action potentials [7]. This was confirmed by Protti *et al.* by the use of goldfish retinal cells. In this experiment, the introduction of TTX to the pipette solution did not cause changes in the cell response, indicating the presence of Ca^{2+} currents rather than Na^+ currents (Figure 4b) [8].

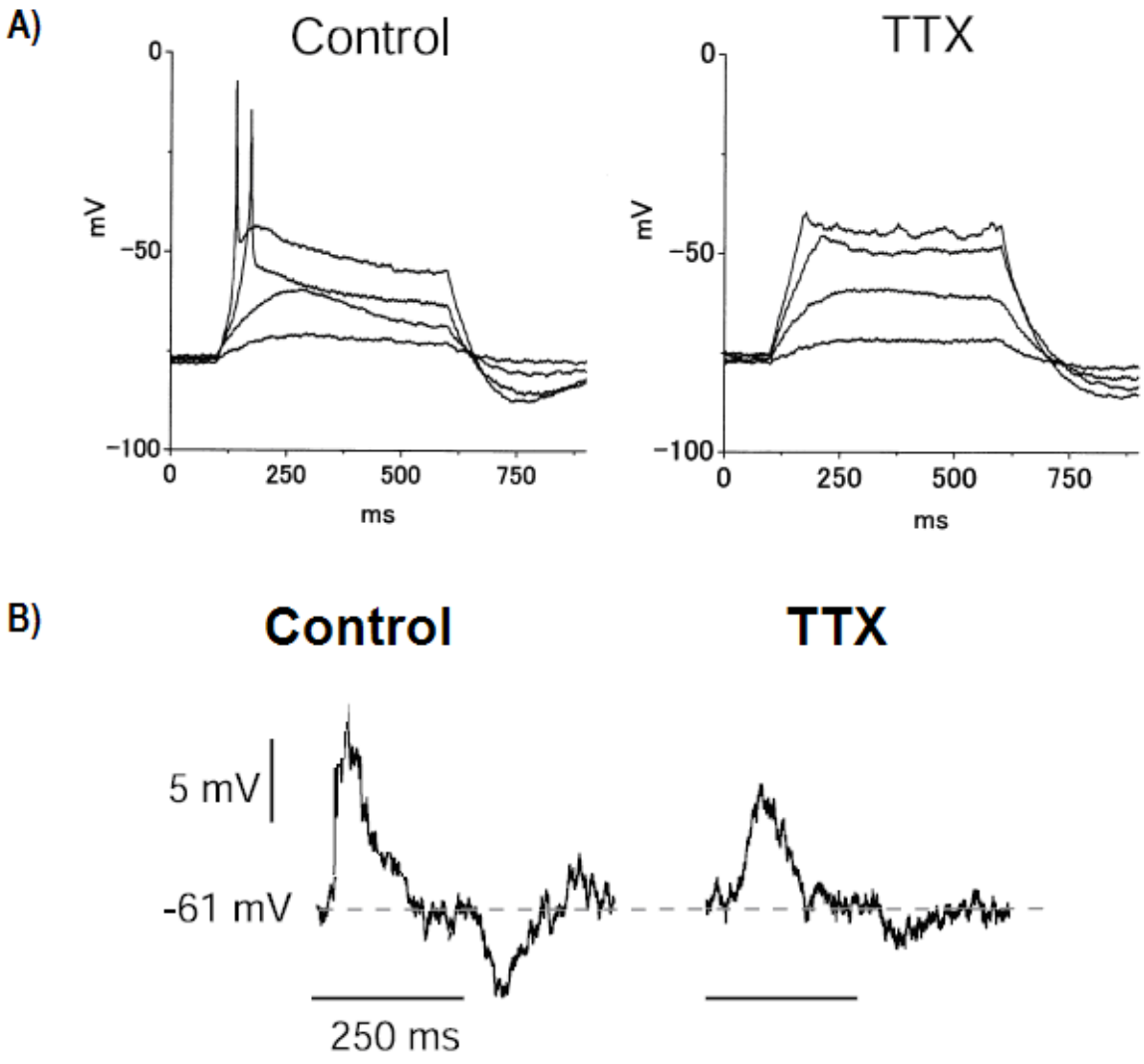

Figure 4: A) In human photoreceptors, the normal action potential (seen in the control) was not generated in the present of TTX. B) No significant decrease in signal strength was recorded in the presence of TTX when goldfish photoreceptors were tested [5, 8].

Currently, the reason for differences in voltage-gated channel types between species is not clear. Variations in the types and the intensities of light that certain groups of animals are exposed to, the activities that depend on photoreceptor function, and the results of other differences in the nervous system could all play a role. A study by Fain *et al*. showed that Na^+ channels cause faster changes than do Ca^{2+} channels; this suggests that the sight of humans and other vertebrates might require a quicker response than is needed in other animals [9]. In addition, it is necessary for some animals to see better in dim light, or to see a specific range of light wavelengths [7]. Whole-cell patch clamping has provided a means to uncover the cellular mechanisms behind these differences and quantify the potential and current changes that take place during photoreceptor function. The use of this technique will persist in this field in order to answer the questions that still remain.

Conclusion

Whole-cell patch clamping will undoubtedly continue to provide insight into the mechanisms involved in photoreceptor function as well as other cell processes. As the technology used for this technique improves, patch clamping will become easier and more accurate. The ability to quantitatively study the flux of ions through a cell membrane has been indispensable in our understanding of cellular activities on the molecular level, which is the basis for fully comprehending activities on a larger scale.

References

[1] DeFelice, Louis. Electrical properties of cells: patch clamp for biologists. New York: Plenum Press, 1997.

[2] Sakmann, B., Neher, E. Patch clamp techniques for studying ionic channels in excitable membranes. Ann. Rev. Physiol. 46 (1984) 455-472.

[3] Bert Sakmann and Erwin Neher. Single-channel recording. 2nd ed. New York: Plenum Press, 1995.

[4] Hamill, O., Marty, A., Neher, F., Sakmann, B., Sigworth, F. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches.

[5] Kawai, F., Horiguchi, M., Suzuki, H., Miyachi, E. Na^+ action potentials in human photoreceptors. Neuron. 30 (2001) 451-458.

[6] Lipton, S., Ostroy, S., Dowling, J. Electrical and adaptive properties of rod photoreceptors in *bufo marinus.* Journal of General Physiology. 70 (1977) 747-770.

[7] Wollmuth, L., Hille, B. Ionic selectivity of I_h channels of rod photoreceptors in tiger salamanders. J. Gen. Physiol. 100 (1992) 749-765.

[8] Protti, D., Flores-Herr, N., Gersdorff, H. Light evokes Ca^{2+} spikes in the axon terminal of a retinal bipolar cell. Neuron. 25 (2000) 215-227.

[9] Fain, G., Gershenfeld, H., Quandt, F. Calcium spikes in toad rods. J. Physiol. (1980) 303: 495-513.