

Science Education Collection

Patch Clamp Electrophysiology

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Abstract

Neuron cell membranes are populated with ion channels that control the movement of charge into and out of the cell, thereby regulating neuron firing. One extremely useful technique for investigating the biophysical properties of these channels is called patch clamp recording. In this method, neuroscientists place a polished glass micropipette against a cell and apply suction to form a high resistance seal. This process isolates a small “patch” of membrane that contains one or more ion channels. Using an electrode within the micropipette, researchers can “clamp” or control the electrical properties of the membrane, which is important for analysis of channel activity. The electrode also allows for changes in the voltage across the membrane, or the flow of ions through the membrane, to be recorded.

This video begins with an overview of the principles behind patch clamp electrophysiology, an introduction to the necessary equipment, and descriptions of the various patch configurations, including whole cell, cell-attached, perforated, inside-out, and outside-out patches. Next, the key steps of a typical whole-cell patch clamp experiment are outlined, in which a current-voltage (IV) curve is generated. Finally, applications of patch clamp recording are provided to demonstrate how the biophysical properties of ion channels, cell excitability, and neuroactive compounds are evaluated in neurophysiology labs today.

Transcript

Patch clamp recording is an extremely useful technique for investigating the biophysical properties of the ion channels that control neuronal activation.

The procedure involves pressing a glass micropipette against a cell in order to isolate a small “patch” of membrane that contains one or more ion channels.

The experimental setup further allows scientists to “clamp” the electrical environment of the patched area by precisely controlling the voltage across the cell membrane, which, depending on the ion channels present, impacts the flow of ions through the membrane and allow for intricate study of these channels.

This video presents an overview of the principles behind the patch clamp technique, a description of the steps necessary to run an experiment, and finally some of the applications of this method.

First, let's review the principles behind patch clamp recording.

The number of positive and negatively charged ions inside a neuron differs from number found on the outside.

This imbalance produces a voltage difference, or membrane potential, of about -70 mV, meaning that the inside is more negative than the outside.

Ion channels help maintain the gradient by controlling the movement of ions across the cell membrane, which are essentially electrical currents.

Using the patch clamp technique, scientists ask questions about the nature of the potential and current.

The patch clamp rig includes a glass micropipette, which contains both an ionic solution and a chlorinated silver electrode for measuring voltages and currents.

The tip of the micropipette has a polished, one micron opening that encloses a small area of membrane.

To eliminate background noise from ions within the bath solution, a high-resistance seal is formed between the pipette and membrane patch. Because the resistance of the seal is in the gigaohm range, it is known as a gigaohm seal.

The electrode within the pipette is connected to an amplifier that can amplifies current and voltage fluctuations that are the result of the movement of ions through channels in the plasma membrane.

With the amplifier, scientists can clamp or artificially set the membrane potential at specific voltages.

The amplifier regulates how much current must be added through the silver electrode in order to keep the voltage constant.

Since different voltage-gated ion channels open at specific voltages, opening events are represented by the variations in the profiles of the measured currents.

Alternatively, scientists can force a specific current through the electrode and record the resulting changes in potential. In this “current clamp” configuration action potentials can be recorded.

Let's now look at the five main types of patch clamp configurations.

First is the cell-attached configuration where the micropipette is simply sealed to the membrane of an intact cell.

Second is the whole-cell configuration where the membrane within the micropipette is ruptured to provide access to the cell's interior.

Third is the perforated patch configuration. Here, chemicals such as antibiotics are added to the micropipette to make small holes in the membrane providing access to the cytosol.

The fourth configuration is the inside-out patch. To achieve this, the micropipette first forms a seal with the cell, then is pulled back quickly, ripping a piece of the membrane off and exposing the inside surface to the bath solution.

This allows for the cytoplasmic side of the channels to be exposed to different chemicals applied to the bath.

Lastly, similar to inside-out, the outside-out patch starts as a whole-cell configuration. The micropipette is slowly withdrawn until a piece of membrane forms a convex seal across the tip.

In this setup, the extracellular face of the channel can be exposed to experimental treatments.

Now that we have reviewed the principles, let's go over the steps required to perform a patch clamp recording.

Start by pulling a borosilicate glass tube into micropipettes using a pipette puller.

Next, fire polish the tip to obtain the appropriate diameter and resistance.

After polishing, fill the micropipette with an ionic solution and flick gently to dislodge any air bubbles.

Then slide the micropipette over the electrode attached to a holder.

Once attached, use a syringe to apply positive pressure to the pipette, which prevents other solutions from entering the tip.

Now, place your cells or tissue of interest on the microscope stage and move the micropipette towards a cell.

With the amplifier generating test voltage pulses, record the resistance, which will increase once the tip is touching the cell.

To form the gigaohm seal, gently switch from positive to negative pressure using the syringe. The formation of the seal will result in a rapid increase in resistance to greater than 1 gigaohm.

Now that a cell-attached configuration has been established, let's convert to a whole-cell configuration and do an experiment!

Recall that a whole-cell configuration is when the membrane is ruptured.

Rupturing is accomplished by adding negative pressure to the micropipette.

Once the membrane breaks, the test pulse shape will have large current transients, as the cell membrane is now acting as a capacitor, which is charged by the test pulse.

The properties of a single ion channel type in any given neuron can be investigated by blocking the activity of other channels types pharmacologically.

A voltage-step protocol is used to examine ion channel currents evoked by stepping the voltage to a series of different holding potentials.

The current-voltage or IV curve shows the voltage-dependences of current flowing through an ion channel and provides insight into at which voltages the channel is open or closed.

Let's now look at a few applications to explore what neuroscientists can do with this technique.

Sometimes, ion channels found in neurons can be studied in a non-cellular environment.

Here scientists have added ion channel proteins to an artificial lipid membrane in order to study those channels in isolation.

These channels can then be exposed to experimental molecules, like hot pepper-derived capsaicin, to study their impact on channel activity.

Because they are exposed to the extracellular environment and significantly impact cellular function, ion channels make excellent drug targets. When test compounds are added to the micropipette or bath solutions, patch clamp recordings can be used to directly test the effect of drugs, like nicotine, on neural activity. The principle of applying negative pressure to form a high resistance seal has even been applied to construct high throughput devices, which can record from numerous cells simultaneously for drug screening applications.

Whole-cell patch clamp is a valuable tool for measuring the response of single cells to stimuli.

Furthermore, paired recordings can be used to investigate the impact of neuron firing on excitable target cells, like muscle. In this example, whole-cell patch clamp is used to stimulate firing of a motor neuron, while recordings are simultaneously taken from the muscle fiber it controls. Clear relationships between neuronal excitation and muscle activity are observed.

You've just watched JoVE's introduction to patch clamp recording where the principles behind the technique and steps in running an experiment were reviewed.

With its exquisite temporal sensitivity to changes in voltage and currents, patch clamp recording will continue to be instrumental in understanding the biophysics of channels and neurons.

Thanks for watching!