Science Lab
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The Patch-Clamp Technique

An Introduction

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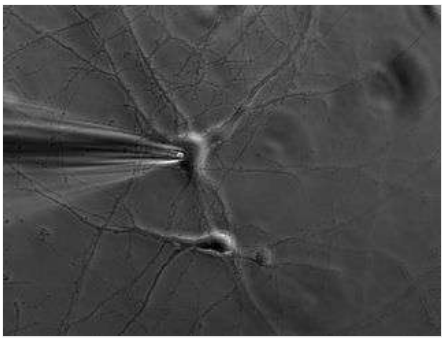
November 09, 2011

Especially in neuroscience, the physiology of ion channels has always been a major topic of interest. The development of the patch-clamp technique in the late 1970s has given electrophysiologists new prospects. It allows high-resolution current recordings not only of whole cells, but also of excised cellular patches. Even single-channel opening events can be investigated. However, with its complex technical, physical and biological background, the need for highly sensitive equipment and the huge amount of skills required of the experimenter, electrophysiology is still one of the most challenging methods in daily laboratory work.



Historical background

Beginning with Luigi Galvani's pioneering work in the 18th century and work by Emil du Bois-Reymond, Johannes Peter Müller and Hermann von Helmholtz in the 19th century, the excitability of membranes and cells has always been of major interest for research on the nervous system. Alan Lloyd Hodgkin and Andrew Huxley revealed the ion channel events of action potentials in 1952 using the voltage-clamp technique, and were awarded the Nobel Prize in Physiology and Medicine in 1963 for their outstanding work.

At this time, voltage-clamp could only be applied to rather big cells as sharp microelectrodes were needed to penetrate the membrane. In the late 1970s, Bert Sakmann and Erwin Neher refined the voltage-clamp technique and for the first time resolved single channel currents across a membrane patch of a frog skeletal muscle. They were also honored with the Nobel Prize in Physiology and Medicine (in 1991). The next breakthrough was the invention of the giga seal by Ernst Sakmann in 1980 which immensely improved the signal-to-noise ratio and allowed the recording of even smaller currents.

Electrophysiology, pioneered in special biophysical laboratories, now expanded to basic biological and medical research and became one of the most important tools for the investigation of the behavior of single cells or whole cellular networks in the nervous system.

General principle

The patch-clamp technique allows the investigation of a small set or even single ion channels. It is thus of special interest in the research of excitable cells such as neurons, cardiomyocytes and muscle fibers.

A single ion channel conducts around 10 million ions per second. Yet the current is only a few picoamperes. Recording currents in this order of magnitude is quite challenging not only for the researcher, but also for the

equipment. In principle, thin glass or quartz pipettes with a blunt end are sealed onto the membrane (Figure 2, 3).

Suction is applied to aid the development of a high-resistance seal in the gigaohm range. This tight seal isolates the membrane patch electrically, which means that all ions fluxing the membrane patch flow into the pipette and are recorded by a chlorided silver electrode connected to a highly sensitive electronic amplifier. A bath electrode is used to set the zero level.

To prevent alterations in the membrane potential, a compensating current that resembles the current that is flowing through the membrane is generated by the amplifier as a negative feedback mechanism (Figure 1).

The membrane potential of the cell is measured and compared to the command potential. If there are differences between the command potential and the measurement, a current will be injected. This compensation current will be recorded and allows conclusions about the membrane conductance. The membrane potential can be manipulated independently of ionic currents and this allows investigation of the current-voltage relationships of membrane channels.

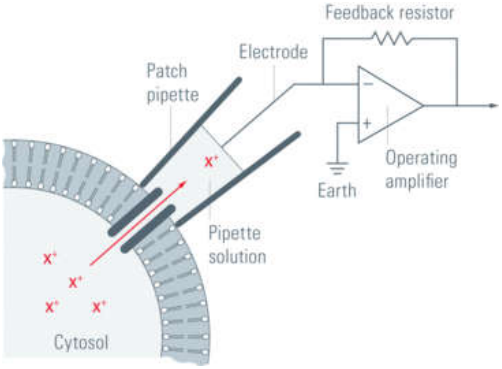


Fig. 1: General principle of patch-clamp recordings. A glass pipette containing electrolyte solution is tightly sealed onto the cell membrane and thus isolates a membrane patch electrically. Currents fluxing through the channels in this patch hence flow into the pipette and can be recorded by an electrode that is connected to a highly sensitive differential amplifier. In the voltage-clamp configuration, a current is injected into the cell via a negative feedback loop to compensate changes in membrane potential. Recording this current allows conclusions about the membrane conductance.

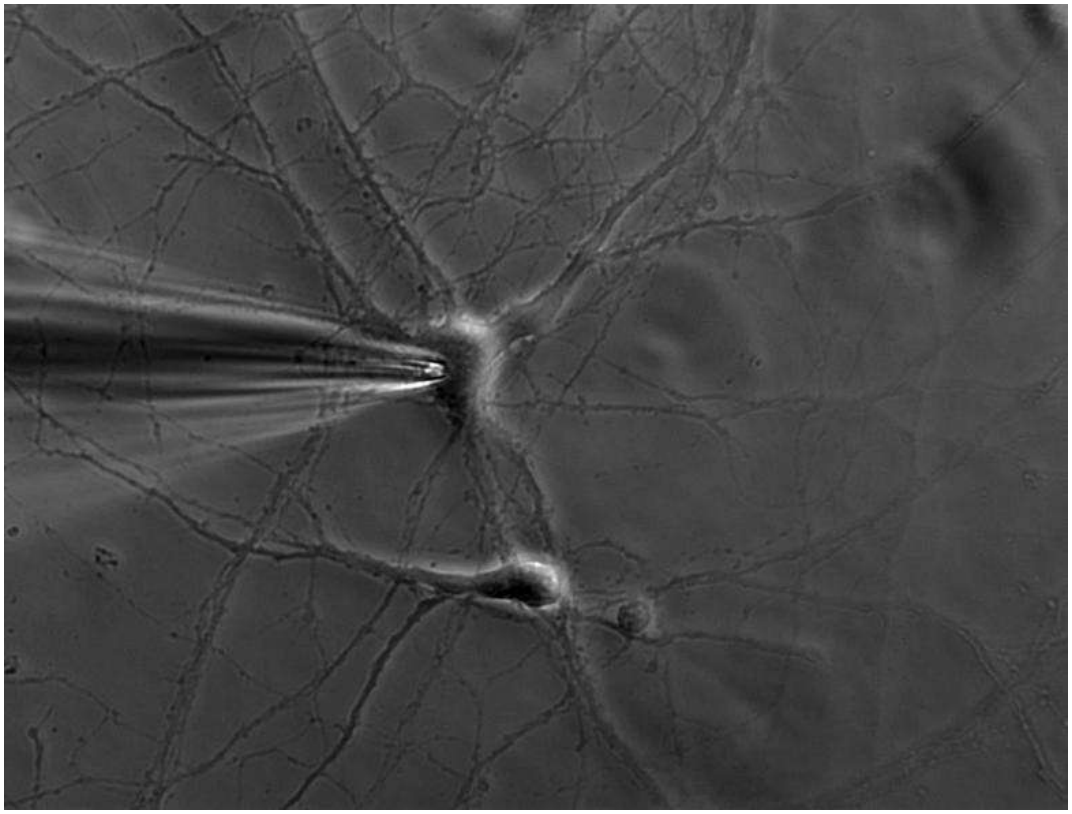


Fig. 2: A phase contrast image of a patch pipette attached to the membrane of a cultured murine hippocampal neuron. Courtesy of Dr. Ainhara Aguado, Ruhr University Bochum, Germany

Configurations

Depending on the research interest, different configurations can be used. In the *cell-attached* mode the membrane patch is left intact (Figure 3). A modification of the *cell-attached* mode is the *loose-patch*. In this case, the pipette is not tightly sealed to the membrane but is only loosely attached to it. This mode is often

used to record action potentials in neuronal cells. The advantage is that the composition of the cytoplasm is not influenced. On the other hand, however, the intracellular environment cannot be controlled.

Applying pore-forming agents (usually antibiotics) via the patch pipette results in a *perforated patch* which guarantees ionic continuity but assures that intracellular proteins are not washed out by the pipette solution. The most commonly used patch-clamp mode is the *whole-cell* mode (Figure 3). To achieve this mode, the membrane patch is disrupted by briefly applying strong suction. The interior of the pipette becomes continuous with the cytoplasm. This method is used to record the electrical potentials and currents from the entire cell. In *whole-cell* measurements the researcher can choose between two configurations: the voltage-clamp mode in which the voltage is kept constant and current is recorded, or the current-clamp mode in which the current is kept constant and changes in the membrane potential can be observed.

Moreover, it is also possible to record currents only from a small patch instead of the whole cell. This raises the chances of recording single channels. The patch can be orientated in two different directions inside the patch pipette. To achieve the *inside-out* configuration the patch pipette is attached to the cell membrane and is then retracted to break off a patch of membrane (Figure 3). In this case the cytosolic surface of the membrane is exposed. This is often used to investigate single channel activity with the advantage that the medium that is exposed to the intracellular surface can be modified.

If the aim is to study the influence of extracellular cues such as neurotransmitters, the *outside-out* configuration (Figure 3) should be chosen. In this case the pipette is retracted during the *whole-cell* configuration, causing a rupture and rearrangement of the membrane. In this configuration the extracellular surface is exposed and thus extracellular cues can easily be applied.

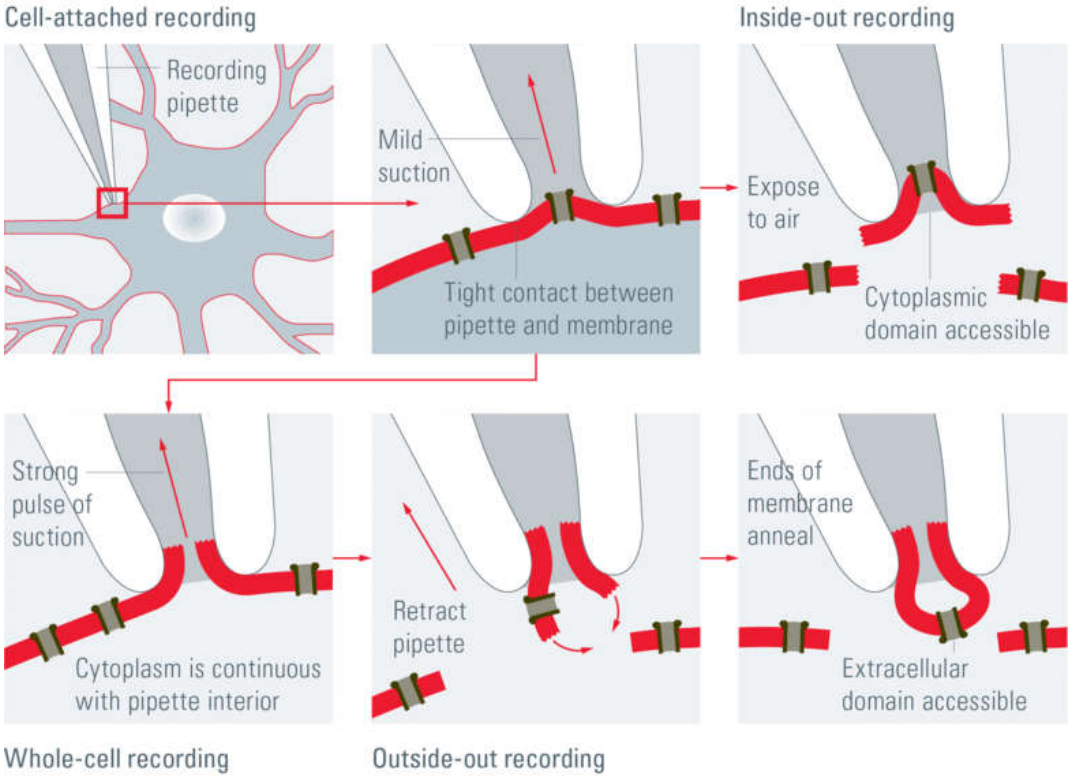


Fig. 3: The four recording methods for patch-clamp: Cell-attached: When the pipette is in closest proximity to the cell membrane, mild suction is applied to gain a tight seal between the pipette and the membrane. Whole-cell: By applying another brief but strong suction, the cell membrane is ruptured and the pipette gains access to the cytoplasm. Inside-out: In the cell-attached mode, the pipette is retracted and the patch is separated from the rest of the membrane and exposed to air. The cytosolic surface of the membrane is exposed. Outside-out: In the whole-cell mode, the pipette is retracted resulting in two small pieces of membrane that reconnect and form a small vesicular structure with the cytosolic side facing the pipette solution. Source: Patch me if you can – What is the patch-Clamp Technique?, puzzledponderer.wordpress.com

Requirements

Patch-clamp experiments can either be performed on cultured cells, acutely dissociated cells or on acute vibratome slices, which allows investigation of the cell's electrophysiological properties in their natural environment. The ion channel of interest can also be isolated and expressed heterogeneously in a common cell line (e.g. HEK293, CHO, LNCaP).

Depending on the sample, either an inverted (cultured cells) or an upright fixed stage microscope (for slices) with a stable platform is needed. If cells in acute slices are investigated, an infrared DIC is recommendable to visualize the membrane. The microscope should be placed on an anti-vibration table because any movement could be fatal to the seal between the pipette and the membrane.

A micromanipulator is needed to move the pipette precisely. Very fine pipettes are formed by heating and pulling small glass or quartz capillary tubes. The diameter of the pipette tip is around 1 µm which encloses a membrane patch that contains only a few or even just one ion channel. The tip of the pipette is heat-polished in a microforge to gain a high-resistance seal onto the membrane. The pipette is filled with a solution that resembles either the extracellular solution or the cytoplasm, depending on the recording mode. The pipette is mounted on a micromanipulator to permit precise movements towards the cell membrane.

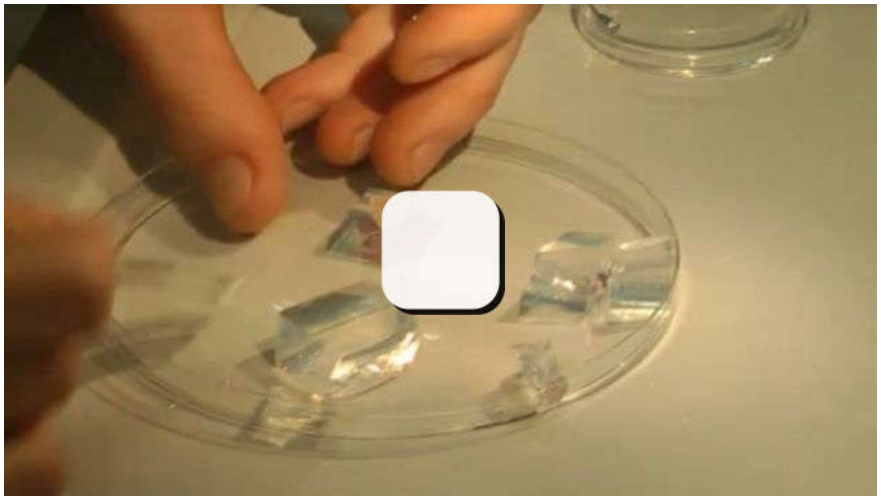
For conductance of the current a chlorided silver wire is used. The bath electrode, which is also a chlorided silver wire, sets zero current value. A differential amplifier with a low-noise transistor is connected to a computer for data acquisition and digitization. Specific software can be purchased to control the amplifier and analyze the data. An oscilloscope can alternatively be used to monitor the currents. If desired, a perfusion system can be added to the setup. Substances can either be applied via a perfusion pencil or by using a POC (perfusion open and closed) chamber.

Applications

Patch-clamp experiments are used to approach a huge variety of physiological questions, not only in neuroscience. During the last two decades patch-clamp recordings have also become more important for the investigation of ion channels in non-excitable cells. It is also a very important method in medical research, since many diseases are related to a malfunction of definite ion channels. In pharmacological research, automated patch-clamping is used to screen potent substances for ion channel modifications.

Patch-clamp recordings can also be combined with live-cell imaging approaches such as Ca^{2+} imaging. In this case a Ca^{2+}-sensitive fluorescent dye is applied to the cell via the patch pipette. The membrane current and changes in fluorescence are recorded simultaneously. Similar experiments can be performed with pH- or Cl^--sensitive dyes.

Video: Preparation of acute brain slices



The video shows the preparation of mouse brain slices using a vibrating microtome. It depicts the whole workflow beginning with the dissection using a stereo microscope, the embedding in low gelling temperature agarose and the slicing itself.

Video: Electrophysiology on acute brain slices

In this video the workflow for performing electrophysiological measurements on acute brain slices using a fixed stage microscope.

Video: Electrophysiology on cultured cells

The video shows an exemplary workflow for performing electrophysiology on fluorescent cultured cells using an inverted microscope. It starts with the preparation of the patch pipettes and ends with the actual measurement on the microscope.

References

1. Areles Molleman, Patch clamping: an introductory guide to patch clamp electrophysiology, John Wiley & Sons Ltd, West Sussex, England, (2003)
2. Bertil Hille, Ionic Channels of Excitable Membranes, Sinauer Associates Inc. (2001)