Voltage clamp and patch-clamp techniques

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Objectives

- Historical background
- Voltage Clamp
- Theory
- Variations of voltage clamp
- Patch-clamp
- Principal
- Patch-clamp configurations
- Applications
- Limitations

1. Introduction

Cells mostly differ in their anatomical, electrophysiological, and gene expression properties, signifying unique functional roles for any given cell type. Much of what we know about the properties of ion channels in membranes of the cell has come from experiments using voltage clamp. In general, the method allows ion flow across a cell membrane to be measured as electric current, whilst the membrane voltage is held under experimental control with a feedback amplifier. The method was first developed by Cole (1949) and Hodgkin and Huxley (1952) for use with the squid giant axon. Since then, many variants of the technique have developed and voltage clamp analysis has been extended to a wide range of tissues.
The effectiveness of the voltage clamp stems firstly from the fact that it allows the separation of membrane ionic and capacitative currents. Secondly, it is much easier to gain information about channel behaviour using currents measured from an area of membrane with a uniform, controlled voltage, than when the voltage is changing freely with time and between different regions of membrane. This is particularly so as the opening and closing (gating) of most ion channels is affected by the cell membrane potential.

2. Historical background

In the eighteenth century Luigi Galvani's pioneering work and work by Bois-Reymond E, Müller J P and Helmholtz H in the nineteenth century, the excitability of membranes and cells has always been of major interest for research on the nervous system. Hodgkin and Huxley (1952) revealed the ion channel events of action potentials using the voltage-clamp technique, and were honoured with the Nobel Prize in Physiology and Medicine in 1963 for their excellent work.
During this time, voltage-clamp could only be applied to rather big cells as sharp microelectrodes were required to penetrate the cell membrane. In the late seventies, Neher and Sakmann advanced the voltage-clamp technique and for the first time determined single channel currents across a membrane patch of a frog skeletal muscle. They were also awarded the Nobel Prize in Physiology and Medicine in 1991. Subsequently the creation of the giga seal by Ernst Sakmann in 1980 which enormously improved the signal-to-noise ratio and allowed the recording of even smaller currents.

Patch clamp technique, pioneered in biophysical laboratories, now extended to basic biological and medical research and became one of the most important tools for the exploration of the of single cells or whole cell behaviour in the nervous system.

3. Voltage clamp

The **voltage clamp** is an electrophysiological technique used by electrophysiologists to measure the ion current through the cell membranes of excitable cells, such as cardiac myocytes and neurons, while holding the membrane voltage at a desired level. A basic voltage clamp will iteratively measure the membrane potential (voltage) and then change the voltage to a desired value by adding the necessary current. This *clamps* the cell membrane at a required constant voltage, allowing the voltage clamp to record what currents are delivered. Because the currents applied to the cell must be equal to (and opposite in charge) the current going across the cell membrane at the set voltage, the recorded currents indicate how the cell reacts to changes in membrane potential. The cell membranes of excitable cells contain many various types of ion channels, some of which are voltage-gated (open and close in response to change in membrane voltage) . The voltage clamp allows the membrane voltage to be manipulated separately of the ionic currents, allowing the current-voltage (IV) relationships of membrane channels to be studied.

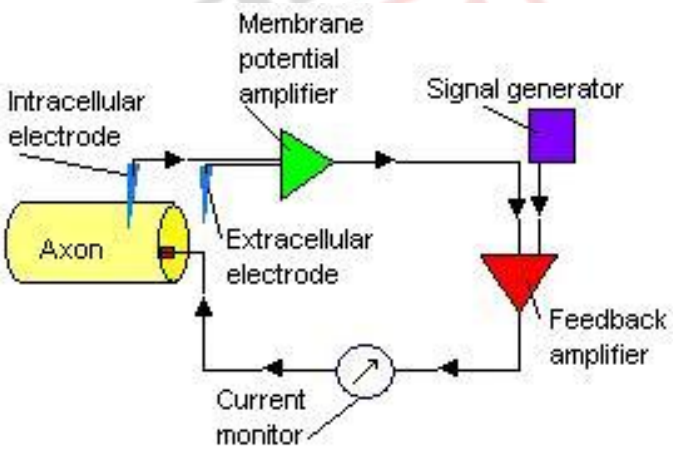

Figure-1. The **voltage clamp** functions by negative feedback. The membrane potential amplifier measures membrane voltage and sends output to the feedback amplifier; this subtracts the membrane voltage from the command voltage, which it receives from the signal generator. This signal is amplified and output is sent into the axon via the current electrode.

3.1 Theory

The basis of the voltage clamp may be understood by consideration of the simplified equivalent circuit for the cell membrane Figure- 2A.
Where
C is the membrane capacity while the channels that allow ionic current
I_i, to flow through the membrane are represented by the variable resistor R
The current I_m flowing through the circuit will be the sum of *I*i and a capacity current,

$$I_m = I_i + C\,\frac{dV}{dt}$$

In voltage clamp experiments the voltage is usually forced to change in a square step manner, being changed as rapidly as possible from one steady level to another (Figure. 2B). Under these conditions a brief spike of capacity current flows at the edges of the pulse, but when the voltage is stable dV/dt is zero and so the capacity current is zero. The ionic current may therefore be obtained free from capacity current once the change in voltage is over. In majority of the experiments ionic current is measured to give information about the permeability properties of ion channels and the mechanisms by which they open and close, though some studies focus on mechanism of the capacity current which is related to channel gating.

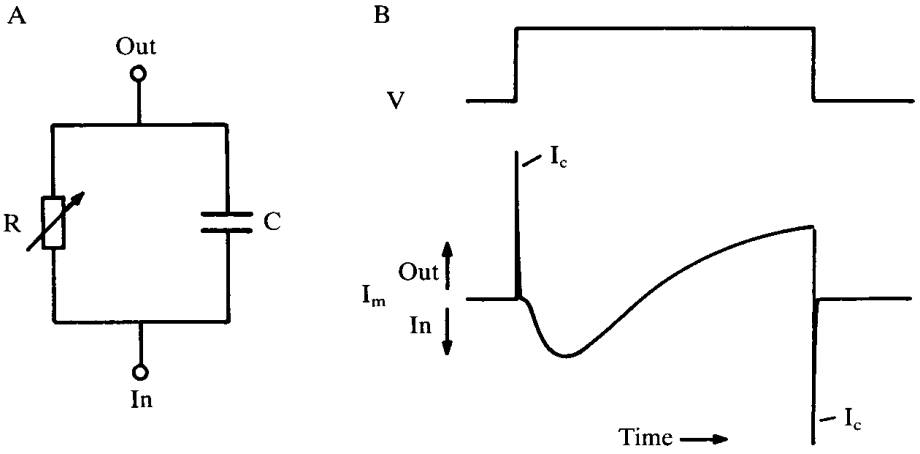

Figure 2. Simplified equivalent circuit for the cell membrane (A). Typical currents recorded from a voltage clamped excitable cell when the membrane potential is stepped in a square manner to a level at which voltage-gated ion channels open. Spikes of capacity current arise at the edges of the pulse; during the pulse inward, and then outward ionic currents flow. The time scale typically in the order of ms (B).

3.2 Variations of the voltage clamp technique

A variety of different voltage clamp methods are given below. The choice of method depends mostly on the size and shape of the preparation to be studied

3.2.i. Axial wire methods

Axial wire technique is applicable to long cylindrical cells such as giant axons of squid and barnacle giant muscle fibres. This method is limited to cells of diameters large enough to permit penetration of the axial wire without damaging the membrane properties.

3.2.ii. Gap methods

Gap methods are also appropriate to elongated cells, though their diameter does not have to be big as it does for axial wires. Eexample, myelinated axons and vertebrate muscle fibres

3.2.iii. Suction pipette methods

Suction pipette methods have been used with preparations that can be dissociated to give isolated cells, for example cardiac myocytes, neurones. The whole-cell configuration of the patch clamp technique uses a pipette with a smaller tip (usually 1 μm) and can be applied to many types of cells which are too small for other voltage clamp methods.

3.2.iv. Two-electrode voltage clamp using microelectrodes

The two-electrode voltage clamp technique is used to examine properties of ion channels. Experimenters use this technique largely to study membrane proteins expressed in *Xenopus* oocytes (Figure-3).

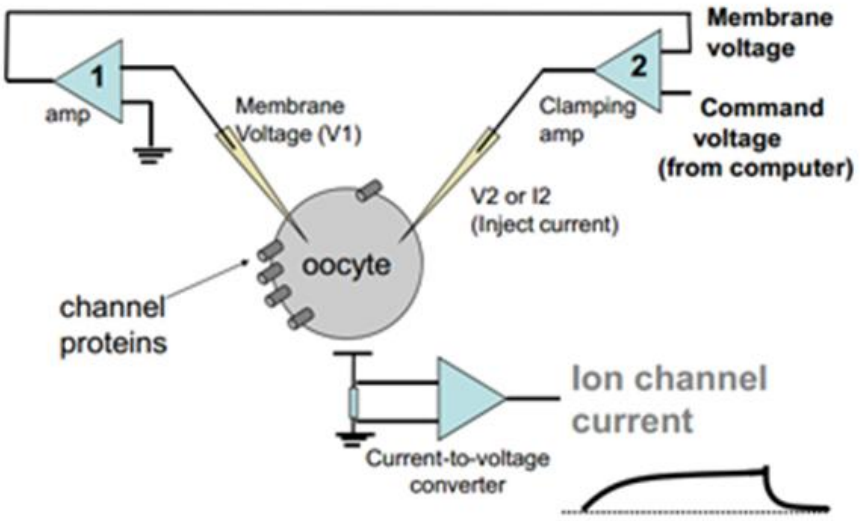

Figure -3. Two-electrode voltage clamp

3.2.iv.a Dual-cell voltage clamp

The dual-cell voltage clamp technique is a specialized variation of the two electrode voltage clamp, and is only used in the study of gap junction channel.

3.2.v. Single-electrode voltage clamp

Single-electrode voltage clamp illustrates a set of methods in which one electrode/pipette is pressed into the cell. This single electrode carries out both functions, current injection and recording. Example. **Patch-clamp technique.**

4. PATCH CLAMP TECHNOLOGY

- Conventional patch clamp technique
  - Patch pipettes
  - Configurations
- High throughput patch clamp systems
  - Automated patch clamp systems

4.1Conventional patch clamp technique

The patch clamp technique is a **specialized version of the voltage clamp**. The patch clamp micropipette has diameter of about 1µ with a polished surface rather than a sharp pointed tip. The patch pipette is pressed against a cell surface and gentle suction is applied to the inside of the pipette to pull the membrane inside its tip. The suction cause the pipette to form a tight seal or Gigaseal with the cell membrane with an electrical resistance of several gigaohms (GΩ) which is essential for high quality recording. Single operational amplifier is used in patch clamp circuit in the 'current to voltage' configuration to control the voltage and

determine the current across the patch. There are various patch clamp configurations for diverse experimental conditions.

In this technique micropipettes are used to form high resistance seals on tiny patches of a cell membrane, thus significantly decreasing the surface area of the membrane and allowing high fidelity recordings of single ion channels.

4.2 Principle

A glass pipette with very tiny opening, containing electrolyte solution is firmly sealed onto the neuronal membrane or any other cell membrane and thus isolates a membrane patch electrically. After the application of a small amount of suction to the back of the pipette, the seal between pipette and membrane becomes so tight that no ions can flow between the pipette and the membrane. Thus, all the ions that flow when a single ion channel opens must flow into the pipette. The resulting electrical current, though small, can be measured with an ultrasensitive electronic amplifier connected to the pipette.Recording this current allows conclusions about the membrane conductance.

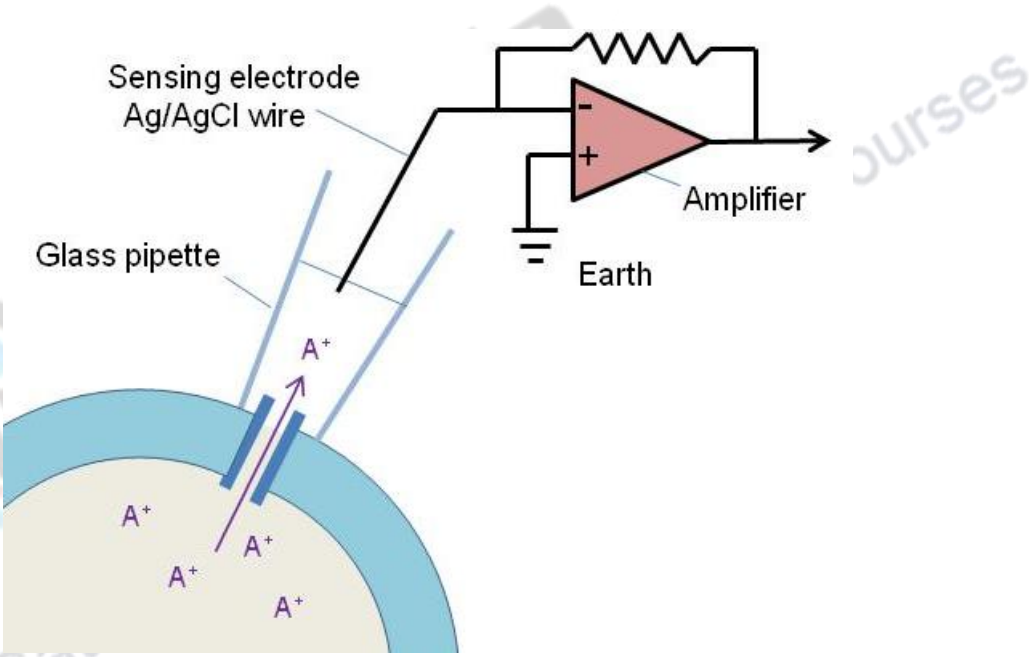

Figure. 4a: General principle of patch-clamp recordings.

4.3 Patch-pipettes

Carefully heating and pulling a small glass or quartz capillary tube, a very fine pipette can be formed. When pulled by machine, the tip will be much smaller than a human hair and the opening on the end of the pipet may be only 1 micron (one-one thousandth of a millimeter) in diameter.

4.3.1.Patch Electrode Fabrication/ pipette fabrication

For any patch-clamp experiment, several steps are essential to make a proper glass pipette. A, a glass that has optimal properties is selected. The properties of glass differ substantially for single-channel recordings and whole-cell-current measurements. For single-channel measurements, low noise is the most important electrical parameter, whereas for whole-cell recording dynamic performance is more important than the contribution of the electrode to the background noise.

4.3.2. General Properties of Pipette Glass

Various general properties of glasses must be taken into account when trying to make best possible pipettes for patch clamping.

- Thermal properties decide the ease with which desired tip shapes can be formed and they determine how easily the tips can be heat-polished.
- Optical properties frequently result in a distinct visual endpoint so that tips can be fire polished the same way each time.
- Electrical properties are significant determinants of the noise the glass generates in a recording condition and determine the size and number of components in the capacity transient following a change of potential across the pipette wall.
- Glasses are complex material made up of various compounds and most of their properties are determined to a first order by the composition of the glass used.

4.3.3. Electrodes Pulling/making

This can be done on any commercially available electrode puller. Modern computerized pipette pullers are capable of pulling glass with almost any thermal properties into the proper blunt-tipped shape that is ideal for whole-cell recording. Therefore, almost any glass can be used to form whole cell pipettes.

4.3.4. Coating of Pipettes

Coating of pipettes with elastomer reduces electrode noise in single-channel recordings. In whole-cell recordings, the noise related with electrode glass is usually insignificant in comparison to other noise sources and so elastomer coating is not often required for noise reduction. Elastomer coating also reduces pipette capacitance.

4.4.5. Fire polishing of Pipettes

To enhance gigohm seal formation and to reduce the chance of tip penetration into the cell during seal formation, electrode tips should be fire polished. Sealing is generally promoted by fire polishing the pipette tip, particularly for cells where seal formation is difficult.

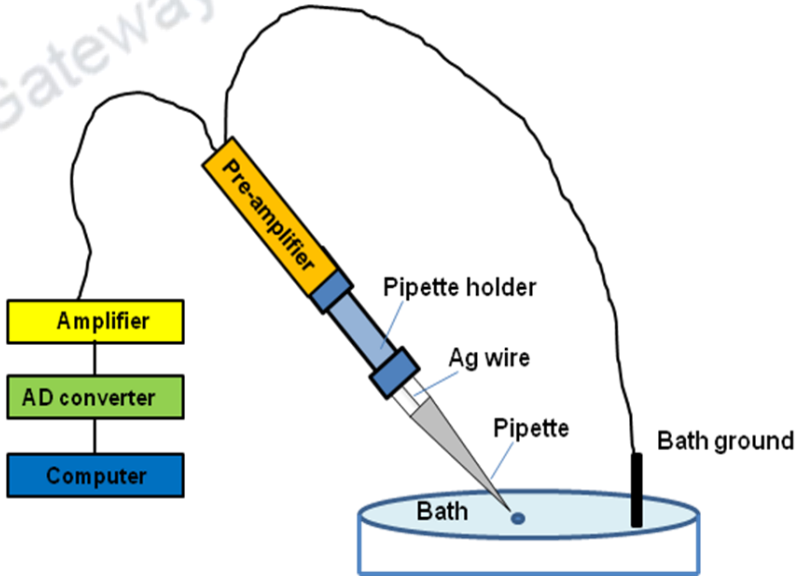

Figure 4b. Patch clamp basic circuit

4.5. Patch-clamp Configurations

- Cell-attached patch
- Whole-cell recording
- Inside-out patch (also called excised patch), giant patches
- Outside-out patch

Each of the four different patch clamp configurations has properties that are useful for studying different kinds of problems **(Figure-5).**

- Recording from cell-attached patches imposes very little perturbation on the cell under study. It allowed single-channel currents nearly all of the known types of ionic channels to be resolved **(Figure-6).**
- Whole cell recordings are routinely used to study electrical currents carried by ions through ion channels, neurotransmifter receptors, and electrogenic transporters in cell types of virtually any origin **(Figure-7).**
- The cell-free configurations inside-out **(Figure- 8)** and outside-out **(Figure-9)** have similar high current resolution and provide control over the ionic milieu on both sides of the membrane.

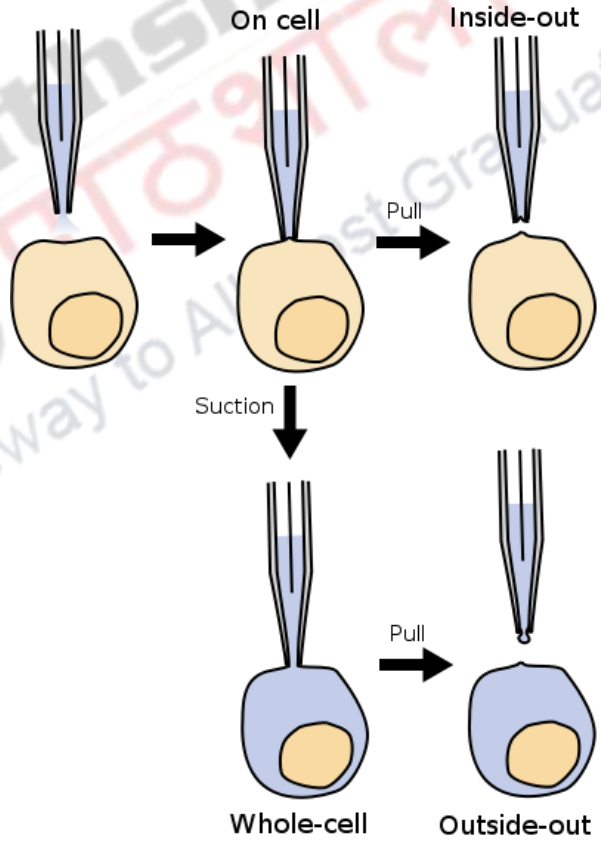

Figure 5 showing variations of the patch clamp technique.

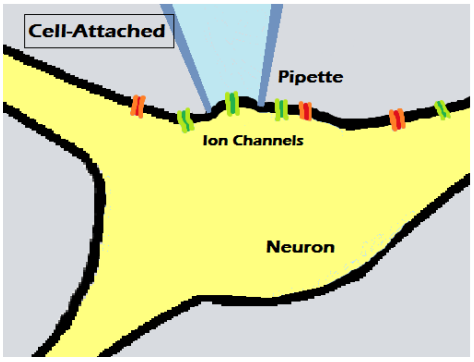

Figure 6. Cell-attached or on cell patch configuration.

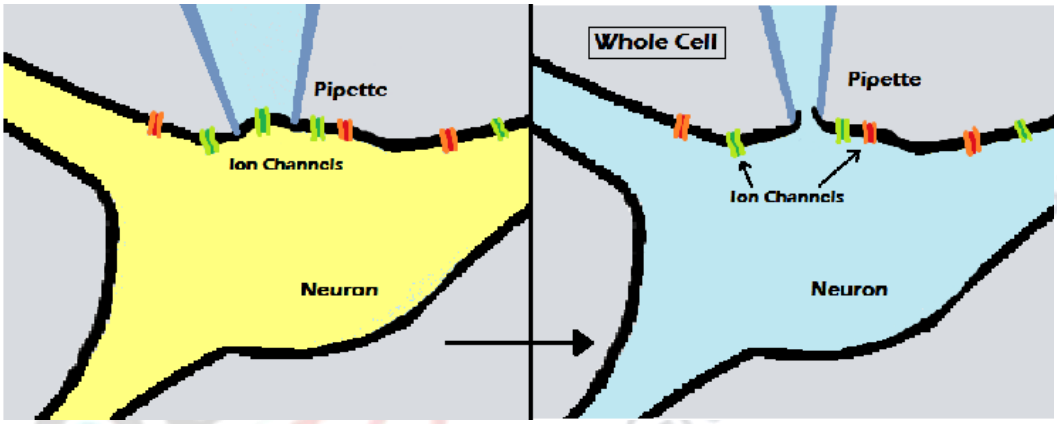

Figure 6. Whole-cell patch configuration.

Figure7. Inside-out patch configuration.

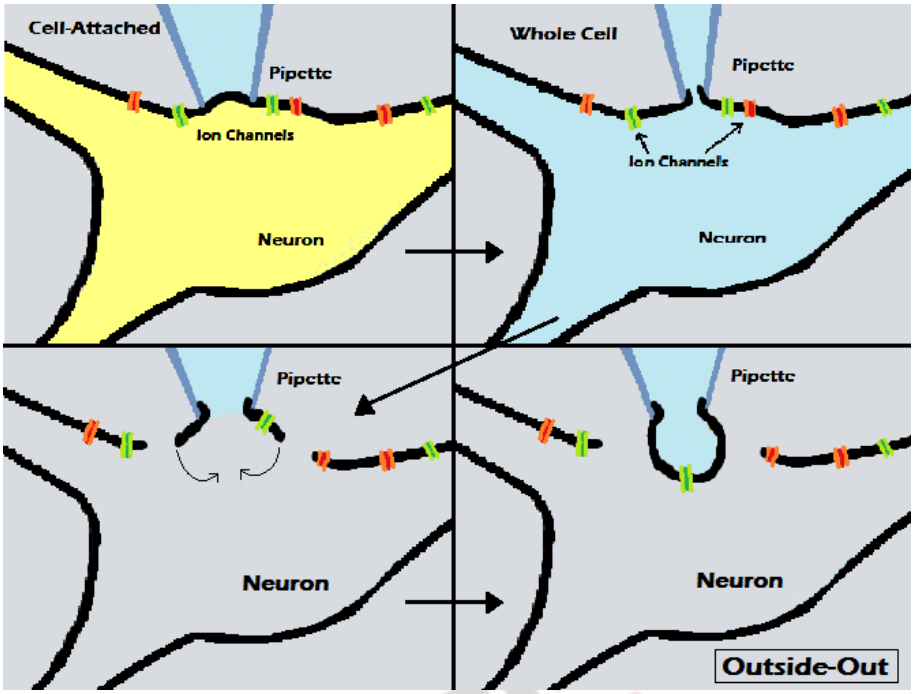

Figure 9. Outside-out patch configuration. In order: top-left, top-right, bottom-left, bottom-right.

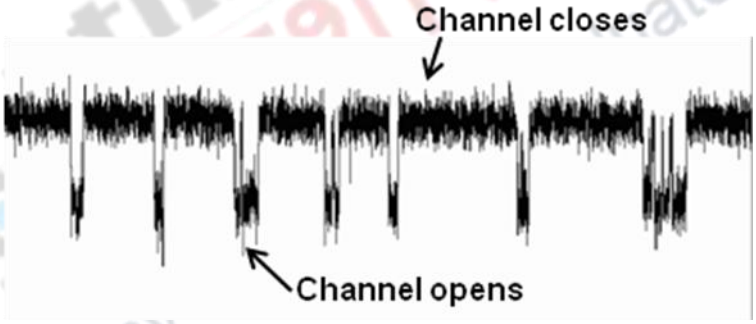

Figure 10. A patch clamp recording of current reveals transitions between two conductance states of a single ion channel: closed (at top) and open (at bottom).

4.5. Automated Voltage-Clamp Technique

Cell membrane receptors and ion channels are among the most significant drug targets, and direct electrophysiological measurement ion currents is the method of choice for analyzing the effects of potential drugs on ion channels and transmembrane receptors. Therefore, a number of automated techniques have been envisioned, using either pipettes or planar substrates, to automate patch-clamp recording from cultured cells that heterologously express a target ion channel. Automated electrophysiological assays are of great importance for modern drug discovery, and a variety of approaches have been developed into practical devices.

5. Applications

Patch-clamp techniques can be used to great effect to help determine the mechanism of action of a compound. This technique is still the golden standard for studying interaction of drug

molecules and ion channel receptors. Patch experiments can provide comprehensive characterization of drug effect on ion channel function more reliably than other indirect methods. Despite its low throughput regarding the number of compounds tested, patch clamp is considered as inevitable in drug discovery.

The great advantage of patch-clamp in cellular physiology is that it allows sensitive and reliable analysis of the electrical activity of cell membranes at the molecular level. Whole cell is the most popular configuration of the patch-clamp technique. It is easy to obtain among all and it allows the use of intra- and extra-cellular recording solutions particularly devise to isolate the ionic membrane conductance of interest, or to intra cellularly apply modulators or drugs. Patch-clamp recording is a primary tool for studying structures and functions of ion channels. It is among the few techniques that allow proteins to be detected at the single-molecule level. The measurement contains much information on the heterogeneity of channel conformations and their dynamic properties.

6. Limitations

The application of voltage-clamp technique to membrane patches and whole cells has allowed the high-resolution electrophysiological characterization of biological membranes and the properties of ion channels that exist in these cell membranes. Despite its widespread application and continuous improvements, voltage clamp/patch-clamp remains a challenging methodology to many researchers because of the requirement to operate fairly sophisticated electronic instruments, the labour intensive and low-throughput operation.

Summary

The voltage-clamp technique is the gold standard for measuring the function of ion channels. The patch-clamp technique has allowed the currents through single ionic channels to be studied from a wide variety of cells. The patch-clamp technique is tremendously powerful and flexible method for studying electrophysiological properties of biological membranes. It allows the recordings of macroscopic whole-cell or microscopic single-channel currents flowing across cell membrane.

The whole cell recordings are routinely used in electrophysiology laboratories to study electrical currents carried by ions through ion channels, neurotransmifter receptors, and electrogenic transporters in cell types of almost any origin. Since the introduction of the patch-clamp technique in 1981 and the subsequent development of commercial amplifiers, this method of intracellular recording has basically replaced sharp electrode recordings, mainly in the study of cultured cells.