Voltage Clamp:

To explain the ‘overshoot’ of the membrane potential to +30 ~ +40 mV observed at the peak of the action potential, Hodgkin, Huxley & Katz formulated the so-called sodium hypothesis. Basically, this assumes that the initial change in the membrane potential only consists of a selective increase in the permeability to sodium that is large enough to dominate the diffusion regime for a short time. In the extreme case one might expect an overshoot of ~ 60 mV (the equilibrium potential $V_{Na}$ for the sodium ions) but never a substantially higher value. They demonstrated that replacement of the extracellular NaCl by choline chloride, glucose or sucrose, which do not penetrate the membrane, resulted in a reduction of the action potential in proportion to the reduction of the extracellular Na$^+$ concentration, whereas the resting membrane potential remained unchanged. Replacement of the normal extracellular fluid by a hypertonic solution having an excess of sodium resulted in an increase in the overshoot of a magnitude that fitted with that predicted by the Nernst equation.

The amounts of Na$^+$ and K$^+$ that enter/leave the axoplasm during the activity of the membrane can be determined by the use of radioactive tracers. For the squid axon $^{[1]}$ the result was a net Na$^+$ entry of about 4 pmol/cm$^2$ (~20,000 ions across 1 $\mu$m$^2$) and a K$^+$ loss of the same amount. However, these experiments did not provide any information on the temporal course of the inward and outward flows of sodium and potassium. If these two oppositely directed ionic movements should generate a change in the membrane potential like the action potential, the first event must be a charging of the membrane’s inside by an inwards-directed current to a positive value (e.g., +40mV) that is followed by an outward-directed current that leads to the repolarization of the membrane back down to ~70 mV. If these currents are carried by Na$^+$ and K$^+$ ions there must be a time lag between the Na$^+$ entry and the K$^+$ outflow.

An attempt to demonstrate such a split-up of the two currents on the propagating action potential would involve almost insuperable obstacles since the membrane potential changes with time all along the length of the axon (in the manner of a wave packet). Consequently, the membrane current is composed partly of ionic currents crossing the membrane and partly of a component used to charge the membrane capacitance $C_m$ to a changing membrane potential ($i = dq/dt = C.dV/dt$). Therefore, to measure the ionic currents a method was required to eliminate the complications arising from the charging of the membrane capacitance. This method is called the Voltage Clamp technique$^{[2]}$.

By means of this technique, the membrane potential can be kept (clamped) to an arbitrary pre-chosen value and then changed almost instantaneously to a new chosen value and clamped at this new value irrespective of the changes in the ionic currents that might follow as a result of:

- Changes in the driving force on the ions in the membrane, and

$^{[1]}$ The squid axon was used because its diameter of 500~1000 $\mu$m allows capillary electrodes to be readily inserted into the axoplasm. Mammalian nerve axons have much smaller diameters (<10 $\mu$m).

- Changes in the membrane’s permeability to one or several ionic species.

Because of the instantaneous potential displacement from one level to another, the membrane capacitance changes charge only at the instant of the membrane potential change. Therefore, the currents that may be measured during the voltage clamp (where dV/dt = 0) are exclusively ionic currents that flow through the membrane. This is equivalent to connecting the axon’s inside and outside with a controllable constant voltage generator. In practice, this is achieved by inserting into the axoplasm an extra electrode (a so-called current electrode) that is connected to an electronic feedback circuit that, despite changes in membrane permeabilities, supplies a current of just the strength and direction to ensure that the membrane potential remains at a given predetermined level. Changes in the membrane current with time at this clamped membrane potential will provide information about the changes in the membrane permeability to the surrounding ions.

Circuit:

The negative feedback control circuit for the voltage clamp method is shown in Fig.1. In addition to the internal potential sensing electrode, a current electrode is also inserted into the axoplasm and connected to the output of a difference amplifier (gain ~1000) which amplifies the voltage \((V_m - V_c)\), where \(V_m\) is the membrane potential and \(V_c\) is the control voltage that can be changed suddenly in well-defined steps. The output of this amplifier, relative to ground potential, has a polarity opposite to that of the input signal \((V_m - V_c)\) and is connected to the internal current electrode. Commencing with the situation where \(V_m = V_c\), if the input signal \(\Delta V = V_m - V_c > 0\) will appear and give an amplified output of \(G(V_c - V_m)\) that will operate to move \(V_m\) back to the situation \(V_m = V_c\). The membrane current, whose action on the membrane potential \((V_m - V_c)\), is now compensated for by means of the electronic feedback, flows away into the external saline bath and is recorded using two external electrodes.

An external potential sensing electrode is placed near to the outer surface of the axon. This electrode is connected, with the internal potential electrode, to the input of a unity gain difference amplifier. The output signal from this amplifier is \(V_m\), the membrane potential. Another electrode ‘a’ is fixed close to the external potential sensing electrode, and both are connected to a difference amplifier ‘A’ that senses the voltage drop \(I_mR_s\), where \(R_s\) is the known resistance of the liquid layer between the two electrodes. In this way, the membrane current \(I_m\) can be measured and recorded.

In Fig.2 the typical time course of membrane current is shown during a voltage clamp experiment. The membrane potential is changed instantaneously from a resting potential of \(-60\) mV to \(+10\) mV. Inwardly-directed current is negative (the initial outwardly-directed membrane capacitance charging current is not shown).

Patch-Clamp Recording:

The most direct way of investigating the functioning of membrane ion channels is to record the current which flows through an open channel, or to measure the changes in membrane potential produced by a imposed current. As already described, the potential across a cell membrane can be measured by inserting a glass microelectrode into the cell and measuring the difference between its recorded potential and one registered by a reference electrode located in
the extracellular medium. The voltage-clamp technique allows the membrane potential to be held (clamped) at a constant value, so that the current that flows through the membrane at any particular potential can be measured. However, some cells are too small to allow their penetration by a microelectrode. Also, the plasma membranes of neurons and muscle cells contain a very large number of voltage-gated ion channels, and the membranes of cells that do not exhibit electrical excitations contain a variety of other types of gated channel. The total current crossing a cell membrane is the algebraic sum of the currents flowing through all of these channels – the functioning of a single ion channel is not possible using the conventional voltage-clamp technique. These issues can be overcome using the patch-clamp technique developed by the 1991 Nobel laureates Erwin Neher and Bert Sakmann.[1]

This technique employs the finding that a clean, fire-polished, glass micropipette pressed against a cell can fuse to its membrane to form a very high resistance seal (≥ 10⁹ Ω) of good mechanical stability. This isolates a small patch of the membrane on the cell and the ion channels it contains can then be investigated through either electrical or chemical manipulation. The high resistance seal means that current can only enter or leave the micropipette through open channels in the isolated patch of membrane. Neher and Sakmann were thus able to report the first recording of the activity of a single-channel (an acetylcholine-activated channel). By applying mild suction, a patch of membrane can be removed from a cell, so that the current through a single channel can be recorded as a function of different compounds exposed to the inside (cytoplasmic) membrane surface of a cell. By increasing the suction, an excised patch can also be prepared having its external membrane surface (outside-out) exposed to an outside solution (Fig.3). Measurements can be made of the millisecond kinetics for membrane ion currents as low as 10⁻¹² A (~10⁷ ions/sec), with voltage clamping to give precise control over channel voltage-gating (Figs 4-6).

**Application to Drug Discovery:**

The specific and regulated functioning of membrane ion channels plays important roles in many physiological processes. These include electrical signalling in the brain and heart, the secretion of hormones into the bloodstream, the transduction of sensory signals, the regulation of blood pressure and immune responses. Defects in ion-channel function can therefore result in profound physiological effects, and more than 55 different inherited ion channel diseases, termed as ‘channelopathies’, have in fact been identified.[3] Ongoing patch-clamp studies of ion-channel function and modulation, coupled with the identification of specific genetic defects that lead to ion-channel related diseases, are providing insights into the relationship between ion-channel structure and function. A well studied example is the potassium-ATP (K<sub>ATP</sub>) channel, where mutations of its pore-forming proteins lead to an impaired ability of ATP to bind to the channel and thus to inhibit the channel’s transport of potassium ions. This can increase K<sub>ATP</sub> channel currents sufficiently enough to reduce electrical activity in nerves and muscles, leading to such diseases as diabetes, epilepsy and muscle weakness. This understanding has led to the development of drugs that specifically block (K<sub>ATP</sub>) channels. The therapeutic action of many existing drugs (e.g., local anaesthetics, sedatives, anti-anxiety and anti-diabetic) is through their

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interaction with membrane ion channels. The following table summarizes some of diseases that are related to ion channel dysfunction (derived from Ashcroft, 2006):

<table>
<thead>
<tr>
<th>Ion Channel</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{K}^+$</td>
<td>Diabetes; Epilepsy.</td>
</tr>
<tr>
<td>$\text{Na}^+$</td>
<td>Epilepsy; Heart; Hypertension.</td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$</td>
<td>Angina; Cardiac Arrhythmia; Epilepsy; Hypertension; Migraine; Muscle Weakness; Chronic Pain.</td>
</tr>
<tr>
<td>$\text{Cl}^-$</td>
<td>Constipation; Cystic Fibrosis; Deafness; Epilepsy; Kidney.</td>
</tr>
</tbody>
</table>

Pharmaceutical companies have in the past faced problems in developing high-throughput assays able to randomly screen their large libraries (typically > 25,000) of potential ion-channel drugs. Many existing ion-channel drugs were developed without knowledge of the precise drug target and mode of action at the molecular level. Although molecular-induced modulation of ion channels can be observed using ion-sensitive or voltage-sensitive fluorescent dyes, for example, this lacks the precision, temporal resolution, and voltage control that can be obtained using patch-clamp measurements. However, conventional patch-clamp studies are too technically demanding and laborious for the primary screening of potential ion-channel drugs. Recently, automated and medium-throughput techniques have been developed (Fig. 6) and are beginning to have an impact on the drug discovery ‘pipeline’ by providing high quality, information-rich, and biologically-relevant assays.