Patch clamp

Excitable cells such as neurons or myocytes express themselves using bioelectrical signals in addition to biochemical ones. Such cells maintain an internal potential 50–80 mV negative relative to the extracellular space (the membrane potential) by the action of the sodium–potassium ATPase (Aidley, 1998).

In addition to sodium and potassium, the concentrations of other ion species such as chloride and calcium are maintained at different levels within the cell relative to that without. These concentration gradients generate electrical potentials, the effect of which can be varied by the opening or closing of ion-specific channels within the cell membrane (Hille, 2001). The actual ion flux that results from channel opening is quite small; however, this is enough to change the membrane potential dramatically (by around 100 mV in the case of a neuron firing an action potential), as well as to trigger biochemical signalling cascades – particularly so in the case of calcium entry.

Recording these signals requires sensitive, specialist equipment (Sherman-Gold, 2008). While the bulk activity of populations of cells can be recorded on a larger scale using techniques such as electroencephalography or magnetic resonance imaging, physiological experiments involving excitable cells commonly focus upon the individual cell as a unit of study.

The first reported intracellular recording of an action potential was carried out by Hodgkin and Huxley (1939) in the squid giant axon. This preparation was chosen for its large diameter (typically ~500 μm), which enabled a microelectrode to be placed inside the axon. Later, in a series of papers (culminating in Hodgkin and Huxley, 1952), an early voltage-clamp method was used to dissect out the ionic basis of action potential generation. This method relied upon placing a second microelectrode within the axon to act as a reference. While this technique was extended to other preparations, such as the Purkinje fibre (Draper and Weidmann, 1951), it remained impractical to introduce two electrodes into smaller cells. Furthermore, such intracellular recordings could only resolve the bulk effect of all ion channels in the cell membrane, providing little detail about the individual characteristics of single ion channels.

The invention of the patch clamp technique has allowed experimenters to record single ion channels, small groups of channels, or intracellular membrane potentials or currents in practically any cell type using only a single electrode. Since its development in the late 1970s (Hamill et al, 1981; see also Molleman, 2002) many variants have been established, and it is now considered the gold standard method for studying the activity of ion channels. The importance of patch clamp and the discoveries that it enabled has been recognized throughout the biomedical sciences, not least by the awarding of a Nobel Prize in 1991 to Bert Sakmann and Erwin Neher. In a medical context, the technique is of particular importance in the study of diseases such as arrhythmias or epilepsy that may arise from dysfunction of these channels, as well as in the identification of potential targets for therapeutic agents.

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Preparations

Patch clamp recordings can be made in a variety of tissue preparations. Isolated cells can be dispersed from a tissue of interest by exposing the tissue to enzymatic digestion. Recordings can also be made from cultured cells or cell lines. These cells can be genetically manipulated to drive or suppress the expression of particular proteins of interest, helping the investigation of channel properties in a controlled environment.

Neural activity in the brain can be recorded using slices obtained by thinly sectioning live brain tissue. Acute brain slices are maintained in vitro to allow for experimentation within a few hours of preparation. Organotypic brain slice cultures can be prepared, useful for observing changes in neural activity over days, weeks or even months.

Patch clamping can also be used to record activity in vivo (Margrie et al, 2002) – including invertebrates, rodents or higher mammals. Such experiments allow for recordings in the intact nervous system, which is particularly useful when studying sensory or motor systems, or behaviour.

Recording configurations

A variety of recording configurations can be used depending on the type of activity the investigator is interested in recording (*Table 1; Figure 1*).

The cell-attached configuration is ideal for recording the activity of single ion channels located in a small patch of cell membrane. This involves bringing a glass pipette to the plasma membrane of a single cell or neuron. Gentle suction is then applied, drawing the cell membrane onto the pipette tip, creating a high-resistance physico-chemical seal. The high electrical resistance of a good seal, typically several gigaohms, allows isolation of small signals arising from the activity of a few ion channels in the patch of cell membrane under the tip.

In a variant of this mode, loose-seal cell-attached, the pipette is brought onto the cell membrane but without forming a so-called 'gigaseal'. This allows the investigator to record the activity of a wider portion of the cell membrane, and is typically used to record isolated action potentials from a single cell.

Ion channels can be studied in isolation by excising a small patch of membrane from the rest of the cell. Following gigaseal

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Table 1. Patch clamp configurations and applications

Configuration	Application
Cell-attached	Recording single ion channels in a patch of membrane of an intact cell
Loose-seal cell-attached	Multiple recordings along different parts of the cell membrane or for recording a larger area of the membrane when compared with cell-attached
Inside-/outside-out	Recording single ion channels isolated from the rest of the cell. Access to the intra- or extracellular domain allows further investigation of an ion channel's behaviour
Whole-cell	Simultaneous recording of multiple ion channels over the entire cell membrane or for membrane potential changes undergone by excitable cells. Introduction of dyes, nucleic acids or other substances into a cell
Perforated patch	Used for intracellular recording when dialysing the cell with the pipette solution is unwanted

formation, the pipette is quickly withdrawn from the cell, tearing away a small patch of membrane, leaving the intracellular surface of the membrane including the intracellular portion of the ion channel exposed to the bath solution, i.e. 'inside-out'. If the pipette is slowly retracted following the formation of a gigaseal, a patch of membrane will become detached and reform into a convex loop around the tip of the pipette with the extracellular domain of the ion channel now exposed to the outside solution, i.e. 'outside-out'.

Alternatively, once a gigaseal has formed on the surface of an intact cell, the membrane under the tip of the pipette can be ruptured using a brief pulse of suction. Under this whole-cell configuration, the intracellular compartment is electrically continuous with the electrode, allowing the recording of post-synaptic potentials or currents and action potentials. Since diffusion will occur across the open tip, dyes, drugs or even nucleic acids (Rancz et al, 2011) can be introduced into the recorded cell by including these in the pipette solution.

However, this diffusion also results in the cell becoming dialysed with recording solution, practically limiting the recording time to <1 hour. Therefore, a perforated-patch configuration can be used to record whole-cell activity when dialysis is particularly problematic. Here, the pipette solution contains small amounts of an antifungal or antibiotic agent, which introduces small pores into the membrane. These pores allow for electrical coupling between the intracellular space and the electrode, but greatly reduce the rate of dialysis. Recordings can therefore be made over a longer timecourse, and without the potential for affecting the internal homeostasis of the cell.

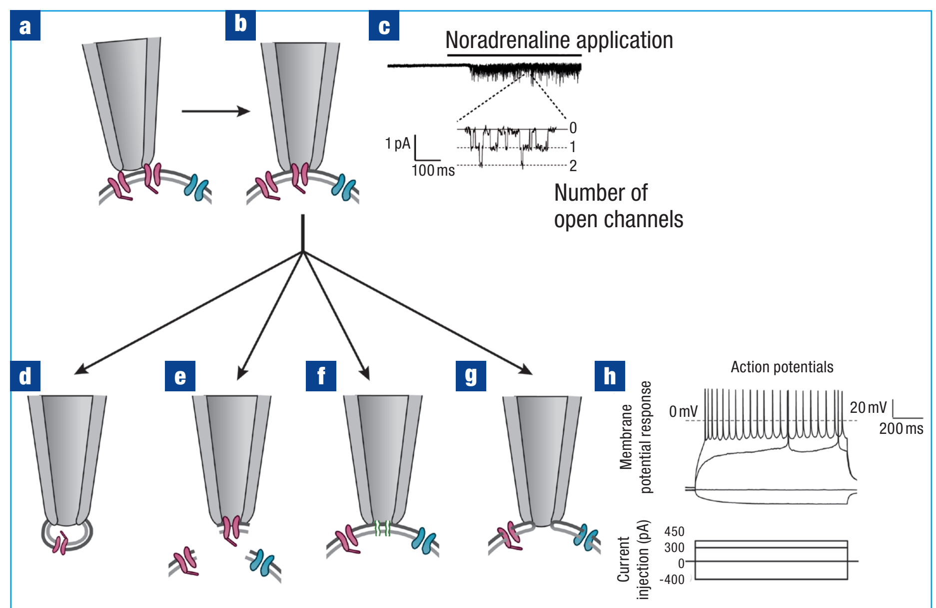

Figure 1. Patch clamp recording configurations. **a.** Loose-seal cell-attached. **b.** Gigaseal formation, leading to cell-attached configuration. **c.** An example recording showing cation currents in a vascular smooth muscle cell in response to noradrenaline application. Individual channel openings are apparent in the enlarged section. From this configuration, a number of others can be developed (data from HG, unpublished). **d.** Outside-out patch clamp. **e.** Inside-out patch clamp. **f.** Perforated patch clamp. **g.** Whole-cell configuration. **h.** An example recording from a mouse LII/III pyramidal neuron, demonstrating membrane potential response to hyperpolarizing and depolarizing current injection steps. Action potentials are evoked by the depolarizing steps (data from AB, unpublished).

Recording mode

Recordings are typically obtained in one of two modes. Current clamp mode applies a constant current at the tip of the pipette, and measures the membrane potential over time. If the current is held at zero, the recording effectively becomes a 'passive' recording of the cell's membrane potential dynamics. Short current injections can be used to mimic synaptic events, and longer injections are used to define the passive electrical and spiking properties of cells, for example to find the minimum current step required to elicit one action potential ('rheobase').

In contrast, voltage clamp mode holds a cell (or patch of membrane) at a specified potential, and measures the current required to maintain that set voltage. By varying the holding potential and measuring the resulting current, the voltage–current ('V–I') relationship can be defined for the channel (or group of channels) under investigation. Since the individual potential gradients of ion species differ according to the Nernst equation, inferences can therefore be drawn regarding the permeability of the channel.

For example, under physiological conditions excitable cells maintain a surfeit

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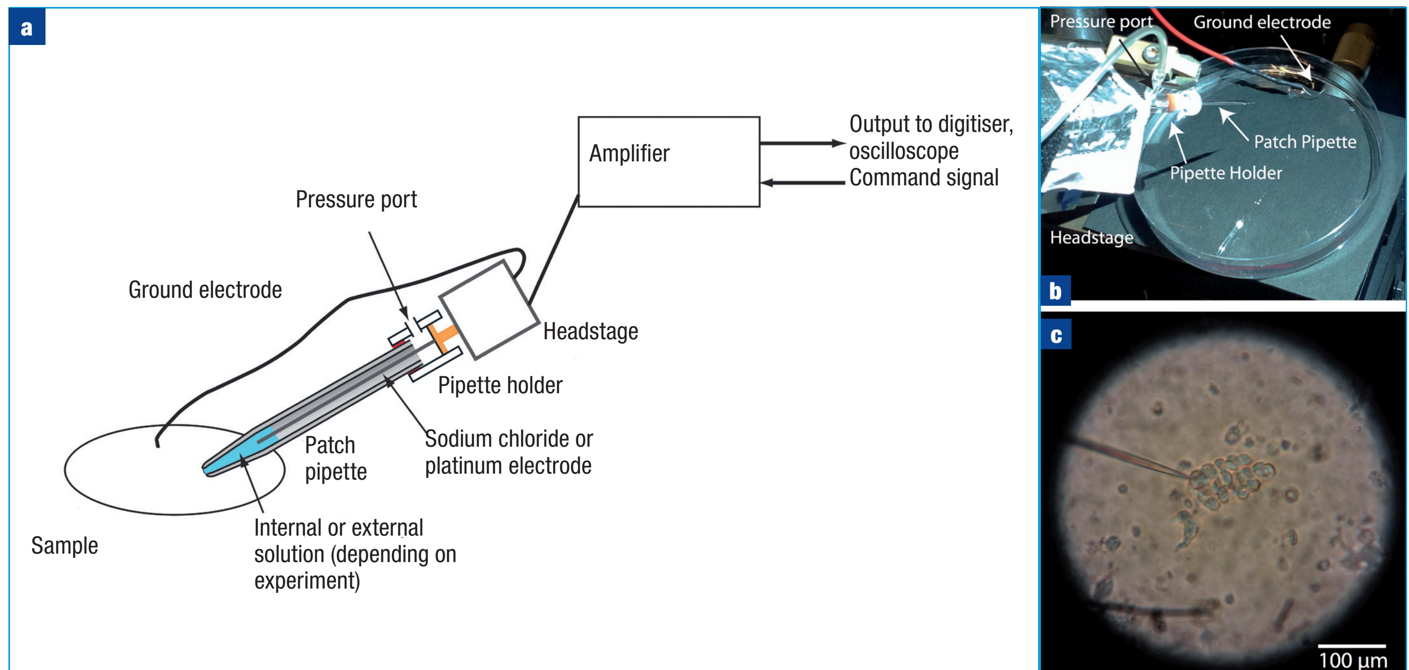
Figure 2. Basic patching setup. **a.** Schematic diagram of essential components (see text for details). **b.** Photograph of a patching setup, showing the same components as in (a). **c.** Light micrograph of a cluster of endothelial cells. A patch pipette recording in cell-attached configuration can be seen to the left of the image.



of potassium ions within the intracellular space, so diffusion will tend to cause K+ ions to move out of the cell, resulting in the inside of the cell membrane becoming negatively charged relative to the external side. For K+, the potential at which no ions would flow is around -83 mV, so a channel that was totally selective for potassium ions would be expected to show no current when held at this potential. This allows calculation of the relative permeability of channels to mixtures of ions.

The selection of voltage clamp or current clamp depends on the type of information the investigator wishes to gather and the preparation from which he/she is recording. Broadly speaking, voltage clamp is ideal for characterizing individual ion channel properties; current clamp is more commonly used when investigating membrane potential changes resulting from stimulation by synaptic events or pharmacological agonists.

Combining the two modes can provide complementary data that make for a more detailed picture. For example recording in current clamp mode can show profound hyperpolarization in an endothelial cell stimulated by acetylcholine. When switching to voltage clamp, the investigator can then observe the activation of the potassium channels responsible for the change in membrane potential observed earlier (Hannah et al, 2011).

Equipment

The equipment needed to perform patch-clamp experiments will vary widely according to the specifics of the preparation and experimental methodology. However, simple electrophysiological equipment can be obtained easily and (relatively) cheaply. The core inventory needed to begin performing recordings (*Figure 2*) includes glass pipettes and a pipette puller, intracellular solution, a pipette holder mounted on a headstage, in turn mounted on a manipulator, an amplifier and analogue-to-digital converter, and suitable software to record data.

- Glass pipettes: typically borosilicate, 1.5–2 mm in diameter
- A pipette puller is used to draw the pipettes to a fine tip suitable for sealing onto cell membranes. Pullers operate by melting a single pipette at the centre, and then pulling the two ends apart to create a pair of patch pipettes, typically to a tip diameter of 1–2 µm. They range from simple manual devices which use electrical filaments to heat the glass and small weights to provide tension, up to fully automated systems incorporating laser heating, air cooling between heating steps and multi-user programmable control. Additionally, some experimenters 'polish' the tips of pulled pipettes using a small butane blowtorch or similar, in order to aid successful seal formation.
- Once pulled, the tip end of the patch pipette is filled with a small volume of solution (see below), and mounted on a pipette holder. This grasps the pipette tightly, and typically incorporates a silver wire coated in silver chloride, providing electrical connectivity onto the internal solution within the pipette, and a port allowing for air pressure to be applied to the pipette.
- The pipette holder is, in turn, mounted upon a headstage, a small pre-amplifier whose specifications will vary according to experimental requirements.
- An amplifier controls the experiment, setting the recording mode and current or voltage at the tip of the pipette, as well as amplifying the resulting signal. Most amplifiers include circuits to compensate for artefactual resistance ('bridge balance) and capacitance ('capacitance neutralization') introduced by the pipette, as well as options to adjust the gain, filtering and other parameters of the recording. Some newer amplifiers are computer controlled, whereas others are manually operated.
- An analogue-to-digital converter links the amplifier to a computer, which streams data to a hard drive using appropriate

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> "Once a seal is obtained, mechanical stability of the experimental setup is critical in order to avoid the pipette 'falling off'."

software. Command signals are sent to the amplifier by a digital-to-analogue converter, usually contained in the same device as the analogue-to-digital converter.
- The pipette and headstage are mounted upon a manipulator, which provides precise sub-micrometer positional control.

These items comprise the core of most patch clamp rigs. Additional equipment will be required to maintain the preparation, such as a temperature-controlled chamber incorporating oxygenation for acute brain slices or suitable anaesthesia delivery and monitoring equipment for in-vivo work.

Other common but optional additions include a good microscope (commonly differential interference contrast or epifluorescence, but also confocal or two-photon systems) to help target the pipette to the preparation, an air table to dampen any mechanical vibrations, and a Faraday cage to shield the system from stray electromagnetic noise.

Medium- to high-throughput recording systems (reviewed in Dunlop et al, 2008) have been developed which incorporate most of these functions and allow one experimenter to record from dozens of preparations. This approach is of particular interest in pharmacological or toxicological screening studies, reducing the number of scientists required to carry out repetitive and laborious experiments, while also reducing the number of animals that may be needed.

Intracellular and extracellular solutions

In-vitro preparations of tissue or single cells are maintained in an extracellular solution (or bath solution) that mimics as closely as possible the extracellular environment in vivo. An experimenter can alter the composition of the bath solution by adding pharmacological ion channel agonists or antagonists or by changing the composition of the free ions in the solution.

Depending on the recording configuration, the contents of the pipette solution may vary too. In cell-attached mode, the components of the pipette solution will be similar to that of the bath. In whole-cell recordings, however, the pipette solution will slowly dialyse the intracellular compartment, and so the pipette solution (referred to as 'intracellular solution') is usually designed to mimic the contents of the cytosolic environment as closely as possible. However, this can also be altered by adding pharmacological agents (Saleh et al, 2006), increasing free Ca^{2+} ions to activate Ca^{2+}-activated channels (Sonkusare et al, 2012), or by adding dyes, stains and indicators. Single cells can even be transfected by dialysing nucleic acid in to the cell (Rancz et al, 2011), allowing for targeted genetic manipulation.

Patching pitfalls

Patching is a difficult skill to master, requiring patience and perseverance. It is important to take great care to maintain tissue viability. A stable, high resistance seal is crucial for maintaining a good recording. In order to achieve this, positive pressure is used to prevent biological material settling on the tip while it is manoeuvred to the recording site. Once a seal is obtained, mechanical stability of the experimental setup is critical in order to avoid the pipette 'falling off'.

The precise size and geometry of the pipette tip will also affect the rate of success. These are set by adjusting the settings on the pipette puller. Different experiments will require appropriate tip specifications; it is best to try to replicate a geometry that is known to work, and then to adjust the precise specifications empirically.

Minimizing the effect of electrical noise is one of the key steps in obtaining good-quality recordings, particularly when isolating small currents from single ion channels. The effect of the gigaseal in isolating such channels helps in this respect; reducing any residual noise using effective grounding techniques and/or a Faraday cage will assist further. **BJHM**

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KEY POINTS

- Patch clamp is a family of techniques used to record electrical activity in a variety of targets, from single ion channels up to whole cells.
- Recordings can be made in voltage-clamp or current-clamp mode.
- Multiple recording configurations are possible, including cell-attached, inside-out, outside-out, whole-cell and perforated patch.
- In the medical context, patch clamp experiments are of particular importance in studying the physiology, pathophysiology and pharmacology of ion channels in health and disease.

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