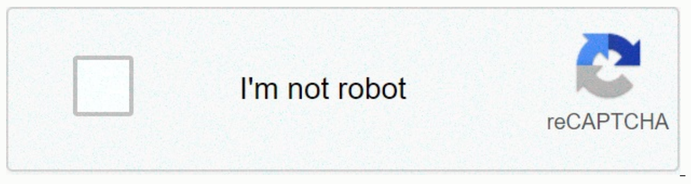

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Whole cell patch clamp technique

A bacterial spheroplast patched with a glass pipette A patch clamp recording of current reveals transitions between two resistance states of a single ion channel: closed (top) and open (at the bottom). Patch clamp technology is a laboratory technique in electrophysiology used to study ionic currents in individual isolated living cells, tissue sections, or patches of the cell membrane. The technique is particularly useful in the study of excitable cells such as neurons, cardiomyocytes, muscle fibers, and pancreatic beta cells, and can also be applied to the study of bacterial ion channels in specially prepared giant spheroplasts. Patch clamping can be performed using the tension clamping technique. In this case, the tension over the cell membrane is controlled by the experimenter and the resulting currents are recorded. Alternatively, the current clamp technique can be used. In this case the current passes over the diaphragm are controlled by the experimenter and the resulting changes in tension are recorded, generally in the form of action potentials. Erwin Neher and Bert Sakmann developed the patch clamp in the late 1970s and early 1980s. This discovery made it possible to record the currents of single ion channel molecules for the first time, which improved understanding of the involvement of channels in fundamental cell processes such as action potentials and nerve activity. Neher and Sakmann received the Nobel Prize in Physiology or Medicine in 1991 for this work. [1] Basic technology Set-up Classic patch clamp setup, with microscope, antivibration table, and micromanipulators During a patch clamp recording, a hollow glass tube called a micropipette or patch pipette filled with an electrolyte solution and a recording electrode connected to an amplifier is brought into contact with the membrane of an isolated cell. Another electrode is placed in a bath that surrounds the cell or tissue as a reference earth electrode. An electrical circuit can be formed between the recording and reference electrode with the cell of interest in between. Schematic depiction of a pipette puller device used to prepare micropipettes for patch clamp and other circuit recordings formed under full-cell or perforated patch clamp The solution that fills the patch pipette can match the ionic composition of the bath ing solution, as in the case of cell-attached recording, or match the cytoplasm, for full cell recording. The solution in the bath solution can match the physiological extracellular solution, the cytoplasm, or be completely non-physiological, depending on the experiment to be performed. The researcher can also change the content of the bath solution (or less common pipette solution) by adding ions or drugs to study the ion channels under different conditions. Depending on what the researcher is trying to measure, the diameter of the pipette tip used may vary, but is usually in the micrometer range. [2] This small size is used to enclose a cell membrane surface or patch that often contains only one or a few ion channel molecules. [3] This type of electrode is distinct from the sharp microelectrode used to puncture cells in traditional intracellular recordings, in that it is sealed on the surface of cell membranes, rather than ingrained through it. Typical equipment used during classic patch clamp recording In some experiments, the micropipette tip is heated in a microsäg to produce a smooth surface that helps to form a high-resistance seal with the cell membrane. To obtain this seal with high resistance, the micropipette is pressed against a cell membrane and suction is applied. Part of the cell membrane is sucked into the pipette, creating an omega-shaped area of membrane that, if formed properly, creates a resistance in the 10-100 gigaohms range, called a gigaohm seal or gigasal. [3] The high resistance of this seal makes it possible to electronically isolate the currents measured over the diaphragm patch with little competing noise, as well as provide some mechanical stability to the recording. [4] Recording Patch squeezing a nerve cell within a disc of brain tissue. The eyedropper in the photograph has been highlighted with a light blue color. Many patch clamp amplifiers do not use true voltage clamp circuits, but instead are differential amplifiers that use the bath electrode to set zero power (ground) level. This allows a researcher to keep the voltage constant while observing changes in current. To make these recordings, the eyedropper of the patch is compared to the earth electrode. Power is then injected into the system to maintain a constant, set voltage. The current needed to squeeze the voltage is opposite in the sign and equal in size to the current through the membrane. [3] Alternatively, the cell may be power-pinched in full-cell mode, keeping current constant while observing changes in membrane tension. [5] Variations Diagram showing variations in patch clamp ing technique Several variations of the basic technique can be applied, depending on what the researcher wants to study. The inside out and out of the out techniques are called ironed patch techniques, because the patch is excised (removed) from the main part of the cell. Cell-attached and both ironed patch techniques are used to study the behavior of individual ion channels in the part of the membrane attached to the electrode. Full-cell patches and perforated patches allow the researcher to study the electrical behaviour of the entire cell, instead of single-channel currents. The full-cell patch, which allows electrical access to the inside of a cell, has now largely replaced high-resistance microelectrode recording techniques to record currents across the cell membrane. Patch Cell-attached patch configuration For this method, the pipette is sealed on the cell membrane to obtain a gigasal, while ensuring that the cell membrane remains intact. This allows the recording of currents through single, or some, ion channels found in the patch of membranes captured by the pipette. By attaching only to the outer surface of the cell membrane, there is very little disturbance of the cell structure. [3] By not interfering with the cell's interior, all intracellular mechanisms that normally affect the canal will still be able to function as they would physiologically. [6] With this method, it is also relatively easy to get the right configuration, and once it is obtained, it is fairly stable. [7] For ligand-gated ion channels or channels modulated by metabotropa receptors, the neurotransmitter or drug being studied is usually included in the pipette solution, where it can interact with what used to be the outer surface of the membrane. The resulting channel activity can be attributed to the medicine used, although it is usually not possible to then change the concentration of the drug inside the pipette. The technique is thus limited to one point in one dose response curve per patch. Therefore, the dose response is achieved using several cells and patches. However, voltage gate ion channels can be fastened gradually to different membrane potentials in a single patch. This results in channel activation as a function of voltage, and a complete I-V (current voltage) curve can be determined in only one patch. Another potential drawback of this technique is that, just as the cell's intracellular pathways are not disturbed, they can also not be directly modified. [7] Inside-out patch Inside-out patch configuration The inside-out method attaches a patch to the eyecupette of the patch, detaches from the rest of the cell, and the cytophoto surface of the membrane is exposed to the external medium, or bath. [8] One advantage of this method is that the test divider has access to the intracellular surface of the membrane via the bath and can change the chemical composition of what the surface of the membrane is exposed to. This is useful when an experimenter wants to manipulate the environment at the intracellular surface of single ion channels. For example, channels activated by intracellular ligands can then be studied through a series of ligand concentrations. To achieve the inside-out configuration, the pipette is attached to the cell membrane, which in cell-attached mode, forms a gigasal, and is then pulled in to break off a membrane patch from the rest of the cell. Pulling off a membrane patch often results initially in the formation of a vesicle of membrane in the pipette tip, because the ends of the patch membrane fuse together quickly after excision. The outer face of vesicle then broken open to enter inside out mode; This can be done by briefly taking the membrane through the bath solution/air interface, by exposure to a low Ca2+ solution, or by temporarily making contact with a drop of paraffin or a piece of hardened silicone polymer. [9] Whole-cell recording or whole-cell piece Hel-cell patch configuration Full-cell recordings involve recording streams through multiple channels at the same time, over a large region of the cell membrane. The electrode is left in place on the cell, as in cell-attached recordings, but more suction is applied to rupture the membrane patch, giving access from the interior of the pipette to the intracellular space in the cell. This provides a means of administering and studying how treatments (e.g. medicines) can affect cells in real time. [10] When the pipette is attached to the cell membrane, there are two methods of breaking the patch. The first is by applying more suction. The amount and duration of this suction depends on the type of cell and size of the pipette. The second method requires a large current pulse to be sent through the pipette. How much power is applied and the duration of the pulse also depends on the cell type. [7] For some types of cells, it is convenient to apply both methods at the same time to break the patch. The advantage of full cell clamp recording over sharp electrode recording is that the larger opening at the tip of the patch clamping electrode provides lower resistance and thus better electrical access to the inside of the cell. [11] [10] One drawback of this technique is that since the volume of the electrode is greater than the volume of the cell, the soluble contents of the cell's interior will slowly be replaced by the contents of the electrode. This is called the electrode dialyzing cell contents. [7] After a while, all properties of the cell that depend on soluble intracellular content will change. The pipette solution used usually approximates the high potassium environment in the cell's interior to minimize any changes this may cause. There is often a period at the beginning of a full cell recording when measurements can be taken before the cell has dialyzed. [7] Outside out patch Outside out patching technique. In order: up-left, up-right, bottom-left, bottom-right The outside-out name emphasizes both the complementarity of this technique to the inside-out technique, and the fact that it places the external rather than intracellular surface of cell membranes on the outside of the patch of the membrane, in relation to the electrode of the patch. [6] The formation of an external patch begins with a holistic cell recording configuration. After the entire cell configuration has formed, the electrode is slowly withdrawn from the cell, allowing a membrane lamp to become bleb out of the cell. When pulled far enough away, this bleb will detach from the cell and reform as a convex membrane at the end of the electrode (like a ball open at the electrode tip), with the original outside of the membrane facing outwards from the electrode. [6] As shown in the picture at the right, this means that the liquid inside the pipette will simulate the intracellular liquid, while a researcher is free to move the pipette and bleb with its channels to another bite of solution. While multiple channels may be present in a bleb of membranes, single channel recordings are also possible in this conformation if the bleb of freestanding membrane is small and contains only one channel. [12] External patching allows the experimenter to investigate the properties of an ion channel when isolated from the cell and is gradually exposed to various solutions to the extracellular surface of the membrane. The trial can examine the same patch with a variety of solutions in a relatively short time, and if the channel is activated by a neurotransmitter or drug from the extracellular face, a dose-response curve can then be obtained. [13] This ability to measure current through the exact same membrane bit in different solutions is the obvious advantage of the external patch in relation to the cellular method. On the other hand, it is more difficult to achieve. The longer formation process involves more steps that can fail and results in a lower frequency of useful spots. Perforated patch Perforated patch technology This variation of the patch clamping method is very similar to the whole-cell configuration. The main difference lies in the fact that when the experimenter forms the gigaohm seal, suction is not used to rupture the patch membrane. Instead, the electrode solution contains small amounts of a antifungal or antibiotic agent, such as amphothericin-B, nystatin or gramicidin, which spreads into the membrane patch and forms small pores in the membrane, giving electrical access to the cell's interior. [14] When comparing whole cell and perforated plaster methods, the whole cell patch can be considered as an open door, where there is a complete exchange between molecules in the pipette solution and the cytoplasm. The perforated patch can be likened to a screen door that only allows the exchange of certain molecules from the pipette solution to the cell's cytoplasm. Advantages of the perforated plaster method, in relation to full cell recordings, include the properties of the antibiotic pores, which allow equilibrium only of small monovalent ions between the patch pipette and the cytosolet, but not of larger molecules that cannot permeate through the pores. This property maintains endogenous levels of divalent ions such as Ca2+ and signaling molecules such as cAMP. Consequently, recordings of the entire cell, such as patch clamping, while maintaining most intracellular signalmechanisms, as in cell-attached recordings. As a result, there is reduced power worn down, and stable perforated patch recordings can last longer than an hour. [14] Cons include a higher supply resistance, relative to the entire cell, due to the partial membrane occupying the tip of the electrode. This can reduce the current resolution and increase the recording sound. It may also take a significant amount of time for the antibiotic to perforate the membrane (about 15 minutes for amphothericin-B, and even longer for gramicidin and nystatin). The membrane under the electrode tip is weakened by the perforations formed by the antibiotic and may rupture. If the patch bursts, the recording is then in full-cell mode, with antibiotics polluting the inside of the cell. [14] Loose patch clamp technique Loose patch clamp is different from the other techniques discussed here in that it uses a loose seal (low electrical resistance) rather than the tight gigasal used in the conventional technique. This technique was used as early as 1961, as described in a paper by Strickholm on the impedance of a muscle cell's surface,[15] but received little attention until they were re-raised and named by Almers, Stanfield and Stühmer in 1982,[16] after patch clamps had been established as an important tool for electrophysiology. To achieve a loose patch clamp on a cell membrane, the pipette moves slowly towards the cell, until the electrical resistance of the contact between the cell and the pipette increases to a few times greater resistance than that of the electrode alone. The closer the pipette gets to the membrane, the greater the resistance of the pipette tip, but if too close to a seal is formed, and it can be difficult to remove the pipette without damaging the cell. For the loose plaster technique, the pipette does not come close enough to the membrane to form a gigasal or a permanent connection, nor to pierce the cell membrane. [17] The cell membrane remains intact, and the absence of a dense seal creates a small gap through which ions can pass outside the cell without entering the pipette. A significant advantage of the loose seal is that the pipette used can be removed repeatedly from the membrane after recording, and the membrane will remain intact. This allows repeated measurements at a variety of locations on the same cell without destroying the integrity of the membrane. This flexibility has been particularly useful for researchers to study muscle cells that they contract under real physiological conditions, get recordings quickly, and do so without resorting to drastic measures to stop muscle fibers from contracting. [16] A major drawback is that the resistance between the pipette and the diaphragm is greatly reduced, allowing leak through the seal, and significantly reduce the resolution of small currents. This leakage can be partially corrected for, however, which offers the ability to compare and contrast recordings made from different areas of the cell of interest. In view of this, it has been estimated that the loose patch technology can solve currents less than 1 mA/cm2. [17] Automatic patch squeezing Automated patch clamp systems have recently been developed, with the aim of collecting large amounts of data cheaply in less time. Such systems usually include a microfluidic disposable device, either a injection molded or a polydimethylsiloxane (PDMS) molded chip, to capture a cell or cell, and an integrated electrode. In a form of such an automated system, a pressure difference is used to force the cells studied to be drawn towards the pipette opening until they form a gigasal. Then, by briefly exposing the pipette tip to the atmosphere, the part of the membrane protrudes from the pipette bursts, and the membrane is now in the inside out conformation, at the tip of the pipette. In a fully automated system, the pipette and membrane patches can then be quickly moved through a series of different test solutions, allowing different test compounds to be applied to the intracellular side of the membrane during recording. [18] See also Bioelectronics Cable theory Channelome Channelomics GHK flux equation Goldman equation Goldman equation Goldman equation Microelectrode array Planar patch Clamp Cutting preparation Refers ^ The Nobel Prize in Physiology or Medicine 1991. nobelprize.org. Nobel Media AB. Retrieved November 8, 2014. ^ Bannister, Niel (1 November 2012). Langton, Phil (ed.). Essential Guide to Reading Biomedical Papers: Recognizing and Interpreting Best Practice. Wiley-Blackwell. doi:10.1002/9781118402184. ISBN 9781118402184. ^ a b c d Sakmann, B.; Neher, E. (1984). Patch clamp techniques for studying ionic channels in excitable membranes. Annual review of physiology. 46: 455–472. doi:10.1146/annurev.ph.46.030184.002323. hdl:21.11116/0000-0000-D552-3. 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