

Bench philosophy (21): Patch clamping guidelines

Successful Patching

Nearly 20 years ago Bert Sakmann and Erwin Neher were awarded the Nobel prize for Physiology and Medicine for their invention of the patch clamp technique. Since then, various forms of patch clamping have become routine, and even labs without an electrophysiology background are kitting themselves out with a patch rig.

It is often said that you need “green fingers” to do patching. I disagree. Successful patching depends on getting a few things right: good quality cells, minimal electrical noise, clean solutions and almost unlimited patience. Put these together and, depending on the cells, you will get those recordings.

Whatever kind of patching you are doing, it all starts with getting a good gigaohm seal. But this first step is often very frustrating. Here are a few rules and guidelines to getting past this first hurdle.

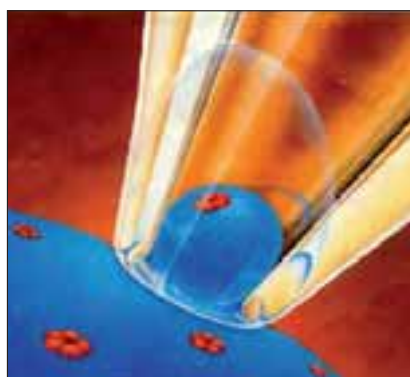
You won't get seals if things aren't clean. Filtering the pipette filling medium is an absolute must. Use 0.22 micron syringe filters. You may even have to filter the bathing medium. Make sure the pipettes are

clean, too. Once you have pulled them, keep them in a box with a lid. And don't touch the middle part of the capillary glass when you are handling them or grease will get on the part that forms the tip. Don't bother trying to save time by keeping pipettes overnight, pull fresh ones each day. Keep a slight positive pressure on the pipette as you approach the cell to blow away any dirt in the saline. And remember you can only cross the air/liquid interface once – all kinds of microscopic flotsam and jetsam are floating on the saline surface, just waiting to hitch a ride on a passing microelectrode tip. So, if your pipette comes back up for air by mistake, get a new pipette.

How to get good seals

You won't get seals if there is serum in the perfusing saline. If you are using cultured cells, that will mean lots of washes in physiological saline to get rid of the serum.

You won't get seals if there is any drift in the pipette whatsoever. This is the most common problem I encounter when people ask me for help in getting seals. You can get away with drifting pipettes once you have formed the seal (after all, that is how you get outside out or inside out patches) but not when the seal is forming. If your manipulator won't hold steady, get a new manipulator.



Trying to get a good seal is the key to successful patch clamping.

You won't get seals if the cells are dead. Obvious, you may think, but sometimes you can't tell, which cells are dead. I spent weeks trying to patch dissociated insect Kenyon cells with no success, not even a scent of a gigaohm seal. I got suspicious and put on some trypan blue, only to find that most of the cells had died. From then on, I put trypan blue on regularly and only tried to seal the cells that excluded the dye. I then started getting seals about 50% of the time – enough to do experiments.

Choosing which cells to patch is crucial to success. Look at the cells under phase contrast. If they have lots of vacuoles they may be unhealthy and there is no way you can patch a sick cell. I go for the ones with bright refringent membranes. But even this is not a hard-and-fast rule: for some cultured cells it is surprisingly the darker ones that patch best.

The calcium concentration in the pipette will have a big effect on seal rates. Calcium ions favour the association between the membrane and glass. The problem is, of course, that if you are going to go whole-cell you will have no choice but to buffer the calcium concentration in the pipette medium to a low value. If you are struggling to get gigaohm seals, it may be worth trying a slightly higher concentration of calcium in the pipette. It may not be any

good for your experiments but at least you will know where the problem lies. I do not know if substituting with another divalent ion like barium or magnesium would work but this may be worth a try.

Patchers use pressure to keep the pipette clean, to help seals form and to break into whole-cell. Play around with the pressure when you are on the cell surface. Some cells seal best with slight negative pressure but, in my experience, if they are going to seal well they will do it without suction. I think of suction as a second-best attempt before giving up. I have even worked with cells that seal under slightly positive pressure. If seals are still reluctant to form, or are forming too slowly, try shifting the pipette-holding potential (under voltage clamp) to a slightly negative value, say about -10 mV. Then, as the seal forms, you can make the potential even more negative.

Be patient

If a seal is forming (as shown by the steady increase in resistance), let it go in its own time. It may just be the cells I have worked with, but I have found the “self-sealers” to be the strongest patches and that makes all the difference when you try to whole-cell. If seals form as soon as you take the positive pressure off, you are either very lucky or you are patching a piece of debris!

In my early days, I was told you cannot use fibre-filled pipettes to do patching. Then an experienced physiologist visiting my lab saw me laboriously back-filling pipettes and laughed. “How quaint,” he said. I have used fibre-filled pipettes ever since. They also told me you have to fire-polish the pipettes. This is not always true and shouldn't be blindly followed. Some cells do indeed seal better with polished pipettes but others seem to seal worse.

Your angle of approach may well be critical in getting a seal. There are different ways of coming up against a cell. Try positioning the pipette tip above the cell and coming down vertically to make contact. Or, if your manipulator allows it, place the elec-



Patch clamp rig with microscope and micromanipulators.

trode above and slightly to the side of the cell and approach along the long axis of the pipette. I have found, I sometimes have the best chance of a seal if I make the approach under visual control and take the pressure off when I see the pipette dimpling into the cell. Other cells have to be approached under “electrical guidance”: watching not the cell, but the square electrical pulses as you advance the electrode. As soon as you see a change in the pulses, take off the positive pressure, sit back and relax while the seal forms. These “instrument landings” work well for many cell types.

If you haven’t got a good seal, you won’t go whole-cell – at least not with electrical properties, good enough to do any experiments with. Good whole-cells need steady gigaseals. I am sometimes asked, which way is the best to go whole-cell. You will have to find that out for yourself because every cell type is different. The only way to find what works for your cells is by trial and error. You have obviously tried varying the negative pressure used to break into whole-cell but also try different ways of applying the pressure. With some cells a steady, constant negative pressure works. With others you have to gradually increase the pressure until the seal breaks. My favourite is popping into cells using little pulses of pressure and the best way to do that is to “kiss” the syringe/pipette tip to which the tubing is attached. I know of one lab that sets up a manometer and applies exactly the same pressure, each time.

Find what method of “popping seals” works for your cell and for you. In all my years of patching, I have never known “zapping” (most amps have a button to deliver an electrical pulse to break the membrane under the seal) to work, at least not on its

own. But I have sometimes found that if I can’t break into a cell with pressure, adding just a little bit of zap to a background of suction does the trick.

The pipette shape matters a lot for whole-cell recording. The rule is to have the tip as wide as you can get away with. Access resistance is your enemy and, in the ideal world, you would use pipettes almost as large as the cells. But although a large pipette pore means good electrical control, it can also mean sucking

cells up into the pipette. Large patches are harder to pull into whole-cell without the membrane breaking and harder to get seals with in the first place. On the other hand, small patches form more easily and need more suction to break in but you get higher access resistance with it.

Go for quality

Once you are in, resist the temptation to start recording straight away. It is not a bad idea, if your recordings are stable enough, to allow a few minutes for the cell interior to equilibrate with the pipette contents. The small ions will equilibrate within seconds. The calcium chelating molecules will take a couple of minutes. If you are doing something fancy like including a large protein to diffuse into the cell, it will obviously take longer. After all, there are a few jobs to do while you are waiting. You don’t just want a whole-cell, you want a “good” quality whole-cell. You can improve the electrical quality by adding a couple more pressure pulses or a bit of negative, or even positive, pressure, all the while monitoring the shape of the square pulse you used to monitor seal formation. Remember, if the ratio of membrane resistance to access resistance is less than ten, you are going to have serious voltage errors. When you have done that, visually check the pipette’s position against the cell because playing around with the pressure can have moved it. Reposition, if necessary.

If you are getting good seals but still not going whole-cell, the angle of approach may be the problem, especially if all that happens is that the seal disappears altogether when you try going whole-cell. If the pipette is touching the side of the cells as you are looking down, you may be pinching the

cell. That will not get in the way of getting a seal but it will certainly put an end to any chances of getting whole-cell.

If you have tried all these tips again and again, got good gigaseals but not any decent whole-cells, it is time for desperate measures. You may have to opt for perforated patching. This technique relies on the fact that certain antibiotics, such as nystatin and amphotericin, punch holes in cell membranes, giving electrical access to the cell’s interior. The idea is to include a saturated solution of antibiotic in the pipette, get a gigaseal and let the antibiotic do the rest. A perforated patch actually has quite a few big advantages. It gives a very low access resistance and the seals are said to be much more stable and, therefore, longer lasting. Another attractive feature is that the pores are impermeable to calcium, so you don’t have to worry about controlling for calcium signalling effects.

So, with all to commend it, why aren’t we all perforating? The reason is simple: antibiotics interfere with gigaseal formation. For advice on how to get around this in detail, the axon guide (http://www.moleculardevices.com/pdfs/Axon_Guide.pdf) is a popular reference but, in essence, the trick is to have antibiotic out of sight while sealing and somehow to have it appear once the seal is formed. In practice, this is usually done by filling the pipette with normal solution near the tip, and backfilling the shank and shaft with antibiotic. The trick is to know how far back to have the antibiotic. Too close and it will diffuse to the tip before a seal has had a chance to form. Too far, and it will get to the seal in about two days’ time. I have to confess that despite several attempts, it has never worked for me – but that may be because I have worked almost exclusively with invertebrate cells.

I cannot give you the final ingredient – this must be cultivated and requires a lot of patience. You can sometimes go for weeks getting nowhere, not even a single decent seal. But eventually it comes and you go another month reeling in data and wondering why you ever found it so difficult. And remember that cells, just like electrophysiologists, can go through bad patches!

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Fancy composing an installment of “Bench Philosophy”?

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