

Bench philosophy (16): 10 tips for better oocyte electrophysiology

The Gift of the Oocyte

The South African frog *Xenopus laevis* is one of the most prominent organisms in today's laboratories. Its lab career commenced as an animal for pregnancy testing in the 1940's and *Xenopus laevis* is currently a crucial model organism for developmental biologists and electrophysiologists. The large eggs spawned by the frog are of special value to researchers. Steven D. Buckingham provides some hints for their optimal use during electrophysiological recordings.

When scientists first toyed with the idea of using unfertilised frog eggs to express protein from messenger RNA, they didn't seriously expect it to work. After all, the oocyte cytoplasm is packed with RNA-degrading enzymes and surely it would be a wonder if enough RNA molecules were to survive long enough to be transcribed into protein. But contrary to all expectations, the procedure did indeed work and today the oocytes of the African Clawed Frog (*Xenopus laevis*) are used by hundreds of laboratories, not only to express proteins but also as a vehicle for assaying the function of ion channels inserted into the oocyte membrane.

Their large size, about 1mm in diameter, is a gift to physiologists. Researchers seeing them for the first time often have to be reassured that, yes, they are indeed looking at one single cell. They are big enough to insert several electrodes simultaneously; furthermore, injecting RNA into the cytoplasm requires the minimum of practical skill and can be accomplished using cheap equipment. This ease of injection gives experimental access to the interior of the cell, as chemicals can be injected into an oocyte during an electrophysiological recording. This is very difficult to achieve with other electrophysiological techniques.

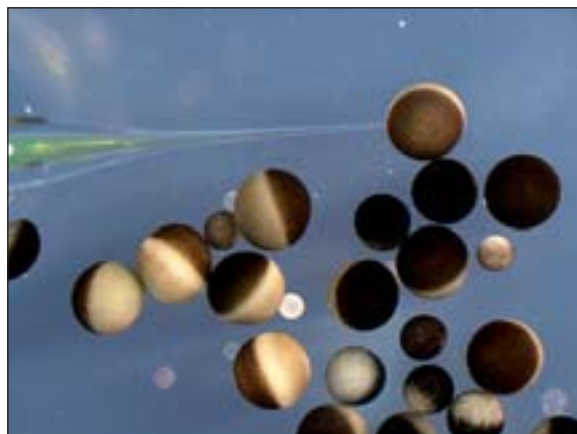
If it is the nucleus you are interested in, again, things couldn't be easier. The nucleus is huge, occupying a significant portion of the animal pole. And to make things even easier, oocytes even come colour-coded, with a brown/black animal pole and a green vegetal pole, so locating the nucleus for injection is easy.

Oocytes as protein factories

Most proteins are expressed within 2-3 days after RNA injection (add an extra day for DNA injections) and continue to be expressed for a further week or so. You don't have tissue culture facilities? Don't worry!

Oocytes are so robust to infection that no tissue culture hoods are needed and injections can be done on an open lab bench.

I have been making electrophysiological recordings from oocytes for over 15 years, specialising in two electrode voltage clamp (TEVC) and patch-clamp. TEVC from oocytes is one of the easiest electrophysiological procedures but can, nonetheless, confound the inexperienced physiologist when things go wrong. Here are a



Size matters! The large size of *Xenopus* eggs makes it easy to inject RNA or chemicals.

few tips that I have gleaned from my years of recording from oocytes, which can improve the quality of recordings and your productivity.

Be fussy with your saline flow. Nearly all oocyte electrophysiology experiments will require changes in the bath solution and this becomes a major issue if you are measuring fast responses to ligands. Although the large size of oocytes is one of their main assets, it makes the fast application of ligands really difficult. You can get around this in some instances by having a local application such as an outlet valve positioned near the oocyte with a background perfusion. For instance, testing the effects of an antagonist or allosteric modulator can perhaps be done by applying the agonist at a known concentration through a lo-

cal applicator, such as a picospritzer, while changing the antagonist concentration in the bath perfusate.

With this configuration, the limitations of quick changes in bath solution don't matter. However, this will not work for concentration-response experiments because they rely on all the receptors "seeing" the same concentration of drug. The only way to obtain accurate measurements of responses to bath-applied chemicals is to get the bath volume to a minimum and the flow to a maximum. By minimal volume, I mean setting the saline level as low as you dare, whilst ensuring the oocyte never touches the surface. Bear in mind that the level may change slightly when you change solutions. In our laboratory, with careful setting up, we can get rapid ligand-gated ion channel responses to bath-applied drugs, reaching a peak within 1s.

Be fussy with your current-injection electrode. Whether you are expressing voltage-gated ion channels (VGICs) or ligand-gated ion channels (LGICs), this electrode has to deliver quite large currents. LGICs are often (hopefully) expressed at quite large levels, so current responses can be quite large. In our studies on nAChRs, we often see responses up to 10s of microamps. In VGIC studies, the large membrane capacitance of oocytes means large currents need to be delivered by the clamp during voltage steps. Any failure of the clamp to deliver these large currents will affect the quality of the recording and, worse, may not be noticed by the experimenter. To deliver these large currents, the current-delivering electrode needs to have as low a resistance as possible. This is accomplished by using a high-ionic strength, pipette-filling medium (typically 3M KCl) and using pipettes with a large tip diameter. This is easiest achieved by pulling low resistance patch pipettes or by breaking back a sharp electrode by carefully driving it against the chamber floor

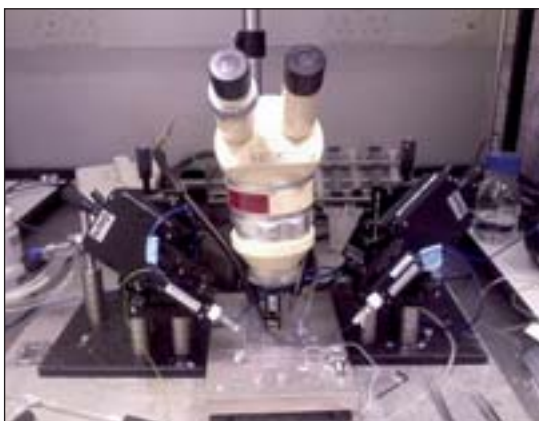
whilst monitoring the resistance. Don't overdo it though: if you can see medium pouring out of the pipette there is no telling what that would do if the electrode were inserted into the oocyte! One way of getting very low resistance pipettes is to fill them with melted agar/3M KCl. Check for bubbles. Insert the two electrodes as far apart from each other as you can, to minimise the risk of poor space-clamp.

Trim the clamp. Increase the gain as far as you can and, if speed of clamp is important (such as when studying VGICs), be fussy with the capacitance cancellation and don't filter the input to the voltage clamp amplifier – this blunder is a common cause of failure to get fast and stable clamp. If you are not interested in particularly fast responses (by fast I mean <10ms as for VGICs) you can make the clamp more stable by increasing the “lag” setting available on many clamps.

Change the oocyte incubation medium at least once every day. Although oocytes are legendary for their resistance to infection, they survive much better (and therefore express more efficiently) if you replace the medium with sterile-filtered medium each day. Some labs find improved survival rates when individual oocytes are cultured in single wells of a 24-well plate but we simply put them into a Petri dish and transfer them daily to a new one containing filter-sterilised culture medium. Some people think that including serum in the medium improves oocyte viability and expression levels.

Once you are getting good expression, put the oocytes in the fridge rather than the incubator. They don't mind the cold and you can prolong their useful life considerably.

Be fussy with defolliculation. Not only does the follicle cell layer have endogenous receptors, which can affect recordings through gap junctions, it can also inter-



Xenopus oocyte rig.



Ready to go: Steven Buckingham just about to start an oocyte experiment.

fere with access of the ligand to the oocyte membrane as well as the uniformity of flow over the oocyte. It is surprising how just a little follicle membrane can affect the time course of responses to bath-applied drugs.

Pay particular attention to expression levels. Everyone has come across problems with expression levels being too low but what about when they are too high? The main problem with very high expression levels, other than difficulties clamping with large currents, is that activation of ion channels can result in large current fluxes. The oocyte membrane has microvilli, which impede the diffusion of ions away from the submembrane space. This will mean that during a large response the reversal potential of the conducting ions will change, leading to what looks like desensitisation. Measurements of kinetics will be erroneous and so will dose-response curves. In addition, many ion channels are modified by intracellular levels of the ions they conduct.

If you want to attempt DNA injections into the nucleus, train yourself by injecting a dye (methylene blue works well) and then dissecting out the nucleus. The dissection is easy – just tear the oocyte apart with a couple of forceps and the nucleus emerges as a ghostly, near-transparent sphere. Some labs gently centrifuge the oocytes before injecting the nucleus, as this brings the nucleus close to the animal pole and you can see its outline. We have not found this step to be necessary, as simply injecting the oocyte with the tip of

the needle placed about the centre of the animal pole hits the nucleus in about 75% of injections.

Egg shortage

Consider alternatives to oocytes. With legislation in many countries getting tighter and rumours of frog shortages being more frequent, supplies of oocytes are already an issue with some labs. Heterologous expression in cultured cells requires more advanced electrophysiological techniques but opens up more possibilities, such as fast perfusion and easier single channel recordings. New transfection techniques, such as ballistic transfection, using devices like GeneGun, can make transfection potentially even less time-consuming than oocyte injection. And, if a lot of experiments are being done on a single receptor construct, it might be worth the effort involved in generating stable cell lines.

Consider investing in a robotic oocyte system. They will never replace the electrophysiologists (at least not yet, thankfully) and they really do work. It isn't just a matter of switching on the machine and going off to do something else but they can be deployed to enhance productivity markedly, especially for medium-throughput screening.

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Fancy composing an installment of “Bench Philosophy”?

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