Video Article

Patch Clamp Recording of Ion Channels Expressed in Xenopus Oocytes

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URL: http://www.jove.com/details.php?id=936
DOI: 10.3791/936

Keywords: Cellular Biology, Issue 20, Electrophysiology, Patch Clamp, Voltage Clamp, Oocytes, Biophysics, Gigaseal, Ion Channels,

Date Published: 10/16/2008

Citation: L Brown, A., E. Johnson, B., B. Goodman, M. Patch Clamp Recording of Ion Channels Expressed in Xenopus Oocytes. J. Vis. Exp. (20), e936, DOI : 10.3791/936 (2008).

Abstract

Since its development by Sakmann and Neher [1, 2], the patch clamp has become established as an extremely useful technique for electrophysiological measurement of single or multiple ion channels in cells. This technique can be applied to ion channels in both their native environment and expressed in heterologous cells, such as oocytes harvested from the African clawed frog, Xenopus laevis. Here, we describe the well-established technique of patch clamp recording from Xenopus oocytes. This technique is used to measure the properties of expressed ion channels either in populations (macropatch) or individually (single-channel recording). We focus on techniques to maximize the quality of oocyte preparation and seal generation. With all factors optimized, this technique gives a probability of successful seal generation over 90 percent. The process may be optimized differently by every researcher based on the factors he or she finds most important, and we present the approach that have lead to the greatest success in our hands.

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=936

Protocol

Part 1: Removing the vitelline membrane

1. Prepare two sets of forceps. We prefer No. 5 forceps, where one set has been slightly sharpened with a file. Also prepare a glass transfer pipette by trimming and fire-polishing a 7" Pasteur pipette.
2. *Recommended:* To prevent the oocytes from slipping, cut a circle of grid (1 mm squares) and glue into the bottom of a 60mm Petri dish. This dish can be reused indefinitely if kept clean between uses.
3. Fill the Petri dish halfway with hyperosmotic solution (see recipe) and place under a dissecting microscope.
4. Remove 1-3 Xenopus oocytes from their incubation solution with a polished Pasteur pipette and place into the dish.
5. Wait 15s - 2 min for cells to begin to shrink. Longer times make cells easier to peel; shorter times lead to healthier cells for patching.
6. As the cell shrinks, the vitelline membrane will begin to become barely visible as a transparent layer over the cell. Select a healthy cell (without whorls or defects) for peeling.
7. With one pair of forceps, gently grasp the vitelline membrane without damaging the plasma membrane. With the other, grasp near the same spot and gently tear the clear membrane apart and free of the cell.
8. With the Pasteur pipette, carefully move the cell to the recording chamber. Note that the cell will be fragile after vitelline removal.

Part 2: Gigaseal generation

1. Fill a recording pipette with saline. Use the minimum volume of solution that makes good electrical contact with the electrode wire in order to minimize pipette capacitance.
2. Flick the pipette several times to allow bubbles to float and slide onto the electrode wire.

   Apply pressure to the filled pipette. This can be done by mouth or with an aquarium pump from a pet store. This pressure is critical to keep the pipette cleans as it crosses the liquid interface and moves to the cell.
3. Find the oocyte in the chamber and focus sharply on the edge of the cell.
4. With the pipette out of the bath, center the (out of focus) tip above the cell. This minimizes time spent in the bath before seal generation.
5. Drop the pipette down into sharp focus next to the cell. Bring it into close proximity (~50 microns). You should see a small distortion of solution pushed out of the pipette by the back pressure.
6. Using the patch clamp software, check the resistance of the pipette (our goal is approximately 3-4 MΩ) and zero the current using the voltage offset.
7. With a positive pressure on the pipette, move the tip slowly towards the cell while monitoring the resistance visually or using an audio monitor that generates a tone whose frequency is proportional to the resistance. As the tip begins to touch the cell, resistance will increase.
8. Suddenly but gently switch from positive to negative pressure of approximately the same absolute value. Resistance should increase to seal formation.
9. The majority of the time, seal formation occurs within several seconds; occasionally 30-60 seconds are required.
10. Once the resistance is at 1GΩ, pressure can be released to neutral. You now have an on-cell patch. For an inside-out patch, pull away from the cell rapidly. For outside-out, see below.

Part 5: Outside-out topology (optional)

1. Immediately after achieving a gigaseal, rupture the membrane. This can be done with sharp suction, but we prefer a 1 V pulse for 1 ms. The resistance should drop to slightly more than the initial pipette resistance.
2. Move the pipette away from the cell slowly and smoothly. You should see a section of the cell pull with the pipette. Suddenly, and within a few seconds, the cell will snap back and the resistance should immediately and simultaneously return to GΩs.
3. You now have a patch in the outside-out configuration. The ectodomains of any included ion channels is exposed to the bath.

Part 6: Anticipated Results

Generally, higher resistance seals are preferable and longer lived. 10 GΩ is a good guideline for high-quality seals. In our experience, the chances of getting seals of this quality vary with many factors, particularly cell and pipette quality. Patch acquisition rates can be over 95% with healthy cells, clean pipettes, and an experienced researcher. These patches may last for many minutes and are suitable for any electrophysiological study of ion channels expressed in Xenopus oocytes, including single-channel recordings.

Discussion

There are many parameters of electrophysiological recording not discussed here. Rig setup, system noise management, channel expression, and recording protocols are all also critical to good experimental results.

In our experience, these are the most critical parameters for forming reliable seals: high quality oocytes, removing the vitelline membrane quickly (a.k.a., "peeling"), use newly pulled pipettes protected from dust, smooth pipettes, positive pressure applied when entering the bath solution, brief times in the bath prior to sealing. This being said, experience varies widely and others have their own sets of most important factors.

Acknowledgements

Shrinking solution recipe is from the lab of R.W. Aldrich. We thank the following funding agencies and foundations for support: National Institutes of Health, National Science Foundation, American Heart Association, Muscular Dystrophy Association, the Donald B. and Delia E. Baxter Foundation, the Klingenstein Fund and the McKnight Endowment for Neuroscience.

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