Video Article

Making Patch-pipettes and Sharp Electrodes with a Programmable Puller

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Abstract

Glass microelectrodes (also called pipettes) have been a workhorse of electrophysiology for decades. Today, such pipettes are made from glass capillaries using a programmable puller. Such instruments heat the capillary using either a metal filament or a laser and draw out the glass using gravity, a motor or both. Pipettes for patch-clamp recording are formed using only heat and gravity, while sharp electrodes for intracellular recording use a combination of heat, gravity, and a motor. The procedure used to make intracellular recording pipettes is similar to that used to make injection needles for a variety of applications, including cRNA injection into Xenopus oocytes. In general, capillary glass <1.2 mm in diameter is used to make pipettes for patch clamp recording, while narrower glass is used for intracellular recording (outer diameter = 1.0 mm). For each tool, the puller is programmed slightly differently. This video shows how to make both kinds of recording pipettes using pre-established puller programs.

Video Link

The video component of this article can be found at https://www.jove.com/video/939/

Protocol

Pulling pipettes

Using a microelectrode puller such as the Sutter P-97 Flaming/Brown, pull a set of approximately 10-20 pipettes.

1. Select your capillary glass. We use borosilicate capillaries (Sutter BF150-110-10, 1.5mm outer diameter, 1.1mm inner diameter, 10cm long). Store the glass carefully so it remains clean and dust free.
2. Design a pulling program. We use a 5 step program, with descending heat and velocity at each step, and a small pull on the final step. Sutter's pipette cookbook is an excellent reference for developing suitable programs.
3. Examine the pipette tips under a microscope to determine opening diameter and smoothness. Discard rough, uneven, or irregular tips. A good pulling protocol should ensure these are rare. For standard patch-clamp recording, tip openings should be 1-3 microns in diameter. A steep taper (blunt tip) leads to lower resistance for the same opening diameter. Shape and size can be modified by pressure polishing.

Fire polishing pipettes

1. Set up a polishing rig (microforge) with a platinum heating filament controlled by a foot pedal. A useful kit is available from ALA Scientific (CPM-2) or a similar apparatus can be assembled piecemeal.
2. *Optional:* Coat the pipette with an insulator to decrease capacitance and improve noise characteristics. We use dental wax. Sylgard 184™ (poly-dimethyl siloxane elastomer also known as PDMS) is also an option.
3. If using wax, keep a small molten supply nearby. With air pressure on the back of the pipette to keep wax from entering the pipette, dip the tip briefly into the liquid wax and remove. With Sylgard, store prepared elastomer in frozen aliquots and paint the pipette tip under a microscope. Heat to cure the elastomer.
4. Place the pipette (coated or not) in the polishing apparatus and bring the tip ~50 microns from the filament. Keep in mind that the filament will expand when heated.
5. *Recommended*: Follow the separate protocol for "pressure polishing" to change the shape of the pipette for optimal resistance and tip diameter.
6. A brief heat pulse (1-2 seconds) is sufficient to remove wax from the tip of the pipette and smooth the glass.
7. Place the finished pipette in a closed box to protect from dust, repeat for 10 successful pipettes.

Discussion

The protocol illustrated here is in daily use in electrophysiology laboratories and is also used to make injection needles for cells and animals. With a programmable puller, it is easy to make pipettes for a variety of uses. With attention and care, the filament on your puller will last for one year or more. Good luck with your experiments.

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