Video Article

Pressure-polishing Pipettes for Improved Patch-clamp Recording

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Abstract

Pressure-polishing is a method for shaping glass pipettes for patch-clamp recording. We first developed this method for fabricating pipettes suitable for recording from small (<3 m) neuronal cell bodies. The basic principal is similar to glass-blowing and combines air pressure and heat to modify the shape of patch pipettes prepared by a conventional micropipette puller. It can be applied to so-called soft (soda lime) and hard (borosilicate) glasses. Generally speaking, pressure polishing can reduce pipette resistance by 25% without decreasing the diameter of the tip opening (Goodman and Lockery, 2000). It can be applied to virtually any type of glass and requires only the addition of a high-pressure valve and fitting to a microforge. This technique is essential for recording from ultrasmall cells (<5 m) and can also improve single-channel recording by minimizing pipette resistance. The blunt shape is also useful for perforated-patch clamp recording since this tip shape results in a larger membrane bleb available for perforation.

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=964

Protocol

Pulling pipettes

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

1. Select your glass. We use borosilicate (BF150-86-10, OD = 1.5 mm, ID = 0.86 mm). Store the glass carefully so it remains clean and dust free.
2. Design a pulling program. The primary goal is a tip opening approximately the desired size, which will depend on application. Sutter's *P-97 Pipette Cookbook* [2] is an excellent reference.
3. Observe your pipettes under a microscope to determine opening diameter and smoothness. Discard rough, uneven, or irregular tips. For standard patch clamp recording, a tip opening that is 2-4 µm across is best. A blunt tip leads to lower resistance for the same opening diameter, which is preferred. Shape and size can be modified during polishing, below.

Pressure polishing pipettes

1. Attach a pipette holder to a clean, compressed air line. Using a regulator, set the compressed air line pressure to ~40 PSI. Connect the compressed air line to a 4-way control valve that will allow the pipette holder pressure to be turned on and off. Connect a pipette holder to the control valve with a length of ⅛ in Tygon® tubing.
2. Use a polishing rig (microforge) with a high-pressure pipette holder, a polishing coil controlled by a foot-pedal, a microscope equipped with a low and high magnification, long working-distance (ideally, 10x and 100x) air objectives. It is critical to be able to observe the pipette opening during the protocol.
3. *Optional*: Coat the pipette with a hydrophobic insulator to decrease capacitance and improve noise characteristics (see "Pulling pipettes" for this technique).
4. Verify the pressure valve is in the off position. Place a pipette into the pipette holder and tighten the fitting to finger-tight.

   Safety note: If the pipette is held tightly into the pipette holder, the air pressure will cause the pipette to be ejected from the pipette holder.

5. Place the pipette and holder in the microforge. Position the pipette holder onto the microscope stage so that both the tip and the polishing coil are visible at low (10x) power on the microforge. Bring the tip ~75 microns from the filament . Keep in mind that the filament will expand when heated.
6. Switch to the high power (100x) objective. Both the pipette tip and the polishing coil should be in focus and in the field of view. If not, adjust the height of the polishing coil. (The pipette's position cannot be adjusted). The pipette tip and polishing coil should be close to opposite sides of the field of view. Avoid positioning the coil too close to the pipette tip because it will expand as it heats and bump into your pipette.
7. Turn the valve to direct pressurized air into the pipette, and heat the pipette tip until the taper behind the tip begins to balloon outwards.
8. Stop heating before the tip balloons too extensively. Experimentation is required to determine the appropriate distance, heat, and time for the desired shape.

9. *Optional*: Reduce the tip opening diameter by polishing the tip in the absence of pressure. Steps 7 and 8 can repeated to fine-tune both the shape and dimensions of the pipette tip.
10. Switch off the pressure and while pointing the pipette tip in a safe direction, remove the pipette and place it in a clean storage box where it will be protected from dust.

Safety note: If the air pressure is not turned off, the pipette can be ejected from the pipette holder during removal.

Notes about the microforge

We use a microforge assembled from off-the-shelf commercially available parts. (You can also buy a pressure-polishing kit from a few companies, e.g., ALA Science, GlassWorx). Regardless of the microforge, it is essential to have optics that are good enough to see the tip opening, which is often only a few µm in diameter. We use a long-working distance 100x lens for this purpose. Also, coat the polishing coil with high-melting temperature glass in order to minimize depositing vaporized metal on the pipettes.

Part 3: Anticipated Results

Moderate pressure polishing can reduce the resistance of a pipette without an appreciable change in opening diameter. If desired, this protocol can be used to make pipettes with sub-micron openings and resistances between 5-10MΩ.

Discussion

We originally developed this method to make pipettes useful for patch-clamp recording from 2-3 µm neurons in *C. elegans* [1]. It is in routine use in the Goodman laboratory. We pressure-polish pipettes not only for recording from tiny *C. elegans* neurons, but also for obtaining macropatch and single-channel recordings of ion channels expressed in Xenopus oocytes. For a given tip-opening diameter, the blunt shape produces a lower-resistance pipette. Lower pipette resistance leads to lower series resistance and lower-noise recordings.

Acknowledgements

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References

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2. Sutter Instruments. P-97 Pipette Cookbook, Rev. D (Sutter, 2008).

