Fabrication of Patch Pipets

Since its introduction approximately two decades ago (Neher and Sakmann, 1976), the "patch clamp" or "patch voltage clamp" technique has revolutionized study of the electrophysiological properties of biological membranes. Originally the name "patch clamp" referred exclusively to the isolation and voltage-clamping of small patches of membrane. The initial objective was usually to observe the tiny currents flowing through individual ionic channels. The most crucial early improvement in the technique was the discovery (Sigworth and Neher, 1980; Neher, 1982) that after pressing the tip of a clean, heat-polished glass pipet against the membranes of certain cells, gentle suction applied to the interior of the pipet often resulted in the formation a membrane-glass seal with resistances well in excess of a gigaohm (10^9 ohm). This phenomenon (usually referred to as a "gigaseal") resulted in large reductions in background noise levels, and within a very short time, a variety of developments made possible by the gigaseal greatly enhanced the versatility of the technique (Hamill et al., 1981). These include the "cell-attached" and "cell-free" configurations, "inside-out" and "outside-out" patches, and the "whole-cell" configuration.

Initially it was believed that the number of cell types that would allow the formation of gigaseals might be quite limited, and tissue culture cells were believed to be best suited for this purpose. In addition, it was thought that the type of glass used was probably important to the formation of such seals. However, as the years have passed it has become obvious that just about any cell type can, with appropriate precautions, form gigaseals; and just about any type of glass, once pulled to an appropriately shaped pipet, can also form gigaseals (although, as will be seen, very large differences in performance not generally related to the formation of the seal are dependent on the type of glass used).

As the technique has improved and its range of applications has grown, the original name "patch clamp" has stuck with it. However, the name now refers to a wide range of electrophysiological measurements, all of which have in common the use of patch pipets and the formation of gigaohm seals. The purpose of this unit is to describe the fabrication of patch pipets. The aspects of the pipet geometry that are important to different applications and the different procedures that have been found to most reliably and simply achieve these results are described. Parameters for glass selection are detailed in Strategic Planning. Pulling patch and whole-cell pipets (see Basic Protocol 1), elastomer coating (see Basic Protocol 2), fire polishing (see Basic Protocol 3 and see Support Protocol 4), pipet filling (see Basic Protocol 4), and pipet testing (see Basic Protocol 5) in an experimental setup are highlighted. Additional support protocols describe alternative ways to optimize pipet geometry (see Support Protocol 1) and cleaning the glass before pulling (see Support Protocol 2). Considerations for noise and dynamic performance are emphasized (see Support Protocols 4, 5, and 6; also see Background Information) as these two requirements for single-channel and whole-cell current measurements dictate how the pipets must be fabricated.

STRATEGIC PLANNING

The major classifications of pipets are those used primarily for single-channel measurements, and those used primarily for whole-cell measurements. The characteristics of pipets that are important for these applications differ in a variety of ways. In the case of single-channel measurements the most important aspects of pipets are often those aimed at reduction of noise. This is because the pipet can be a major (often dominant) source of background noise in this case and because background noise is often the limiting factor in the amount of information that can be gleaned from single-channel measurements.

Contributed by J.L. Rae and R.A. Levis

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Pipets for whole-cell recording are generally fabricated somewhat differently. The type of glass used, the geometry of the pipet, and the elastomer coating, are generally not very important to final noise performance except in so far as they influence the access resistance into the cell. Thus the objective in this case is to produce relatively larger-tipped pipets that minimize pipet resistance. This will also minimize series resistance artifacts and minimize noise at bandwidths above a few hundred hertz.

Patch Pipet

Tubing dimensions

Single-channel and whole-cell current measurements are sufficiently different in their requirements that different factors are important when selecting the proper glass from which to make pipets. For single-channel measurements, noise considerations dominate, and pipets are constructed so as to minimize background noise. For geometry, this means using the shortest possible pipets with the largest possible wall thickness. For practical purposes, glass tubing of ~2.7 in. (6.9 cm) in length is easily handled and the roughly 1.35-in. (3.43-cm) pipets (after pulling) can be fit under the objectives or condensers of most microscopes. Even shorter pipets will result in lower noise recordings, but they become increasingly difficult to handle and to fit under microscope optics. Outside diameters (o.d.) of 1.5 to 1.7 mm with inside diameters (i.d.) of 0.75 to 0.85 mm (o.d./i.d. = 2.0) are also convenient and easily obtainable. Ratios of o.d./i.d. = 3 or 4 are also possible to obtain but with such glass it is much more difficult to place a reference electrode into the small bore of the pipet. This is because as the bore gets smaller, the internal wire used as a reference electrode also gets smaller and more flimsy. When the bore is very small, a reference electrode can be inserted only with great difficulty and might require use of a microscope. Many investigators settle on o.d. = 1.65 mm, i.d. = 1.15 mm (o.d./i.d. = 1.43) as a compromise between low noise and convenience. In addition, an i.d. >1 mm allows the use of commercially available 1-mm-o.d. silver/silver chloride pellet electrodes which never require rechloriding.

Choice of glass type

For low background noise, it is necessary to select glasses with low dissipation factors (Rae and Levis, 1992a: Levis and Rae, 1993). Today, most of the potentially useful glasses with low dissipation factors are no longer available and likely never will be in the foreseeable future. In fact, really only three choices remain: Schott 8250, Schott 8330, and quartz. Pyrex and Kimax glasses are also readily available but they have poorer electrical properties (see Table 6.3.1). As of 2003, 7052 and EN-1 (Kimble's equivalent of 7052) are still available from existing stocks but will not be once those stocks are depleted. Quartz is clearly the best pipet glass in terms of noise and has the added benefit

Table 6.3.1 Glass Properties

Glass	Dissipation factor	Dielectric constant	Softening temperature (°C)
Corning 7052	0.0026	4.9	710
Kimble EN-1	0.0026	4.9	710
Corning Pyrex	0.005	5.1	825
Kimble KIMAX	0.005	5.1	825
Schott 8250	0.0022	4.9	720
Schott 8330	0.0037	4.6	820
Amersil Quartz	10^{-4}	3.8	1580

that it has almost no impurities that could leach from the glass and alter channel gating or conductance. Quartz is available from two manufacturers: General Electric and Heraeus Amersil. Any other glass is expected to have such impurities but whether or not these leach sufficiently to alter channels must be determined by experiment. For ease of pulling, it is useful to select a glass that softens at ≤800°C. Alumina silicate glasses (potentially useful for low-noise recordings) soften at temperatures near 900°C and limit the lifetime of the platinum filaments used as heaters in most pullers. Corning 1724 alumina silicate is available for those who wish to try it. Quartz is the best glass for low-noise single-channel recording, but it softens at ~1600°C and can only be pulled with an expensive laser-based puller. All glass types tested to date are capable of forming gigaohm seals and any suggestion that one glass seals better than another has been purely anecdotal. In addition, if a particular glass seals exceptionally well to a particular cell type, it is unlikely that it would perform the same with all cell types. It is important not to use glass that has an internal filament fused to the wall. While this glass fills easily and seals without difficulty, the internal filament serves as a pathway for filling solution to create an internal fluid film. This internal film can be a significant noise source.

Noise

The electrical properties of the glass used for fabricating patch pipets are of considerable importance in determining patch-clamp noise performance. Probably the most important characteristic in terms of noise is the **dissipation factor** of the glass (see Choice of glass type). This should be selected to be as low as possible. In addition, the **dielectric constant** (see Levis and Rae, 1993) of the glass should also be as low as possible. These two factors combined with the geometry and wall thickness of the pipet and the type and extent of elastomer coating (see Basic Protocol 2 and Support Protocol 6) are the determinants of the **dielectric noise** of the pipet. Dielectric noise has been discussed extensively elsewhere (see e.g., Levis and Rae, 1992; 1993). For a single dielectric, the power spectral density of dielectric noise, S_d^2 (in amp^2/Hz), is given by:

$$S_d^2 = 4kTDC_d(2\pi f)$$

and the root-mean-square (rms) noise, i_d (in amp rms), in a bandwidth B (Hz) is given by:

$$i_d = (4kTDC_d c_2 \pi B^2)^{1/2}$$

where k is Boltzman's constant, T is absolute temperature, D is the dissipation factor, and C_d is the capacitance of the lossy dielectric (here the pipet): c_2 is a factor which depends on the type of filter used to establish the bandwidth B ($c_2 \cong 1.3$ for an 8-pole Bessel filter with a –3 dB bandwidth B).

C_d is determined by the dielectric constant of the glass, the thickness of the pipet wall, and the depth of immersion of the pipet. It is also influenced by the type and thickness of the elastomer coating used. C_d is minimized by a low-dielectric-constant glass, thick-walled pipets, and shallow depths of immersion, all of which are important to low-noise recording. Of course, the magnitude of the dielectric noise produced by the pipet depends on the product DC_d and is thus minimized by small C_d and the lowest possible dissipation factor, D. Elastomer coating is also important to dielectric noise (see Basic Protocol 2 and Support Protocol 6).

Of all glasses, quartz has by far the lowest dissipation factor ($D = \sim 10^{-5}$ to 10^{-4}); it also has the lowest dielectric constant (~3.8). It is thus the best selection for the lowest possible noise recording situations. The dissipation factors of other glasses recommended here are at best some 10 to 30 times higher than that of quartz (see Table 6.3.1). Soda lime glasses have even higher dissipation factors ($D \cong 0.01$) and should be avoided in situations where low-noise single-channel recordings are desired.

Sources of noise other than dielectric noise associated with the pipet are given considerable attention in this unit (see Support Protocols 4, 5, and 6). In general, however, these other noise sources do not significantly depend on glass type. Instead, they are more easily influenced by pipet geometry and elastomer coating (see Background Information).

Capacity transient

As described in detail in Rae and Levis (1992a), the electrical properties of pipet glass are also important determinants of the details of the capacity transient associated with changing the patch potential. The more lossy the glass (i.e., the higher its dissipation factor), the larger will be the slow component of this transient. This can interfere with many types of measurements. Most commercial patch clamps contain circuitry that allow partial compensation for these effects, but none of them are capable of completely canceling the capacity transients arising from lossy glasses such as soda lime glass. Once again, quartz is superior to other glasses in this regard, but good performance can be achieved with low-loss glasses such as 8250 and 8330. Elastomer coating also helps reduce the capacity transient but will be considered below.

Whole-Cell Pipets

Tubing dimensions

For whole-cell recordings, high-frequency noise is dominated by the noise resulting from the series combination of the pipet resistance and the whole-cell capacitance. The dissipation factor and the dielectric constant of the glass usually are not very important in this application. In fact, the only way to optimize whole-cell current recording noise is to make the lowest possible resistance pipet. The resistance of the pipet resides mostly in the region near the tip, and so the length of the shaft of the pipet is not very important. However, if both single-channel and whole-cell measurements are performed with the same setup, it is inconvenient to make single-channel pipets of one length and whole-cell pipets of a different length. Usually the placement of the chamber, the geometry of the microscope, and the length of the pipet and holder dictate the placement of the micromanipulator. Given the limited movement possible with most fine micromanipulators, it would not be possible to use pipets of grossly different lengths without also repositioning the micromanipulator. Thus, as for single-channel pipets, ~2.7-in. tubing lengths are recommended.

To produce the lowest-resistance pipets, one wants the largest possible internal tip diameter. Therefore, for a given outside diameter, the thinnest possible wall is desired. A 1.65-mm-o.d. and a 1.3-mm-i.d. is about what is easily possible. A good compromise for both single-channel and whole-cell current measurements is 1.65-mm-o.d. and 1.15-mm-i.d. Again, a 1-mm commercially available silver/silver chloride pellet can be used as the internal electrode rather than a chlorided silver wire.

Choice of glass type

A reasonable choice for whole-cell pipet glass is Schott 8250, which has a softening temperature of ~720°C, a dissipation factor of 0.0022, and a dielectric constant of 4.9. Schott 8330 would also work well but has a slightly higher softening temperature (820°C). It is still easily pullable on any commercially available puller. It is unlikely that quartz pipets would be required for whole-cell recordings since quartz's excellent electrical properties are largely irrelevant for macroscopic recordings. However, it might be useful should the cells contain a conductance that is sensitive to impurities from glass. Impurities would be expected to be minimized with quartz.

Noise

The electrical properties of glasses used to make whole-cell pipets are generally of much less importance to overall noise performance than is the case for single-channel patch pipets. The reason for this is that the dominant source of noise in whole-cell voltage clamping arises from the thermal voltage noise of the resistance in series with the cell membrane (R_s, normally dominated by the resistance of the pipet) and the capacitance, C_m, of the cell membrane (see Levis and Rae, 1995). This noise source will be described in greater detail (see Support Protocols 4, 5, and 6, and Background Information). Here it is sufficient to note that at bandwidths above a few hundred hertz, this noise is usually significantly higher than that of the patch clamp amplifier and the pipet combined, provided that reasonable precautions are taken not to allow the pipet noise to get out of hand. For example for an access (series) resistance of 5 MΩ and a cell membrane capacitance of 30 pF, this noise source produces ~1.3 pA rms in a bandwidth of only 1 kHz, which is more than five times higher than the typical noise of a whole-cell patch clamp amplifier at this bandwidth, and it is more noise than that produced by low-noise patch pipets at bandwidths ~50 times higher.

Nevertheless, it is still important to pay moderate attention to good low-noise practices in terms of pipets even in whole-cell situations. In the case of glass selection, this simply means avoiding the lossiest of glasses such as soda lime. There is essentially no reason to ever select a high loss glass when other glasses (e.g., Schott 8250, Schott 8330, Kimax, and Pyrex) with much superior electrical properties are available and which have melting points only somewhat higher than the thermally soft but lossy soda lime glasses. At one time, high-lead glasses like KG-12 or 0010 were best for whole-cell recordings due to their very low melting points and versatility of fire polishing. However, new laws about lead glass disposal are quickly making these glasses unavailable.

The most important thing to achieving low noise in a whole-cell voltage clamp situation is to minimize the product R_sC_m. For a cell of any particular size (and capacitance), this means minimizing R_s, and this in turn means pulling pipets with relatively large tips and blunt tapers. This is most easily achieved with glasses with relatively low melting points and thin walls.

PULLING SINGLE-CHANNEL AND WHOLE-CELL ELECTRODES WITH AN AUTOMATED PULLER

BASIC
PROTOC

Automated microprocessor-based pullers allow heat, pull strength, and certain timing parameters to be varied under program control. Others also allow pressure of a cooling gas jet to be altered, although not entirely under program control. A typical way that a pull cycle works with these pullers is as follows: first the heat is turned on. As the glass is heating, the velocity with which the two ends of the glass are separating is monitored. When a program-selectable velocity criterion is met, the heat is turned off, the gas jet is turned on for a program-selectable duration and, following a brief delay, the pull is activated at the strength selected by the program. A full program as recommended here uses four such cycles to finally cause the two pieces of glass to separate.

This protocol describes an approach to optimizing the pipet puller program (see Table 6.3.2) that works for a widely used puller, the Sutter P-97, but it should represent the general approach used with other pullers. This puller and some others have the ability to gate a burst of gas against the glass for cooling. This capability (although not essential) greatly enhances the ability to achieve the desired tapers in the region of both the tip and of the shank. The following procedure works quite well with all glass types but quartz.

Materials

Pipet glass (Garner Glass)
Pipet puller (Sutter Instrument)
Micropipet storage jar (World Precision Instruments)

1. Run ramp test for the electrode puller to determine the temperature at which the electrode glass softens (see manufacturer's instructions) and use the heat determined for the pulling program.

2. Set up a one-line program:

   heat = ramp value
   pull = 5
   velocity = 10
   time = 25.

   Set pressure to 500 (middle of range).

3. With pipet glass in place, start program and see if glass pulls in four loops.

   If only three loops are required to pull the pipet, decrease velocity by one and repeat until glass is pulled on fourth loop. If it takes more than four loops, increase velocity by one and repeat until glass is pulled on fourth loop.

   The authors use four loops because the general shape of the pipet is largely determined by the first three. The fourth loop can then be modified as needed to affect primarily the region right near the tip.

4. Make an identical three-line program with:

   heat = ramp value
   pull = 5
   velocity = value determined above
   time = 25.

Table 6.3.2 Sutter Pipet Puller Programs

Line	Heat	Filament	Velocity	Delay	Pull
Program 1 P-2000 Quartz					
1	960	3	40	130	50
2	875	4	40	130	40
Program 2 P-2000					
1	960	3	40	130	50
2	875	4	40	130	100
Program 3 P-97 Schott 8250, Corning 7052, Kimble EN-1					
1	519	5	10	25	
2	519	5	10	25	
3	519	5	10	25	
4	519	10	50	100	
Program 4 P-97 Schott 8330, Kimax, Pyrex					
1	566	5	10	25	
2	566	5	10	25	
3	566	5	10	25	
4	566	10	50	100	

5. Add a fourth line to the program where:

   heat = ramp value
   pull = 10
   velocity = 50
   time = 100.

6. Pull an electrode and check tip under a high-magnification microscope (600 to 1500×). Adjust the pressure and repeat this step until the desired tip size is achieved.

   If tip is too large, decrease pressure by 100. If tip too small, increase pressure by 100.

7. Place electrode into the micropipet storage jar.

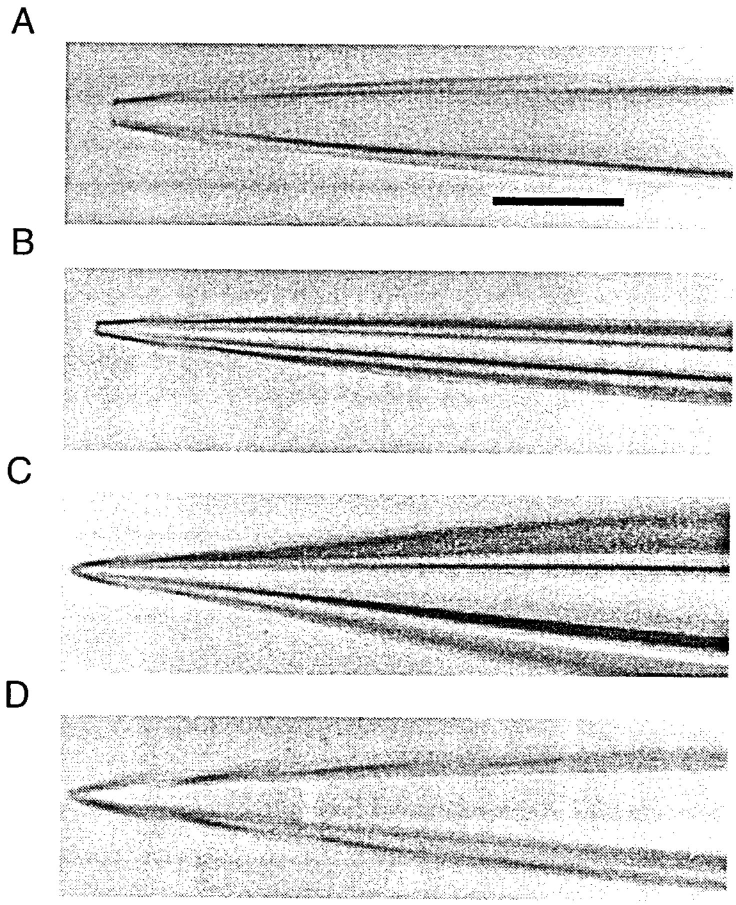

Figure 6.3.1 Single-channel tips before fire polishing. (**A**) Schott 8330 o.d. = 1.65 mm, i.d. = 1.15 mm, Sutter Program 4, pressure = 300. (**B**) Schott 8330 o.d. = 1.5 mm, i.d. = 0.375 mm, Sutter Program 4, pressure = 300. (**C**) Quartz o.d. = 1.5 mm, i.d. = 0.375 mm, Sutter Program 2. (**D**) Quartz o.d. = 1.5 mm, i.d. = 0.75 mm, Sutter Program 2. Tips shaped like those in (C) and (D) would require no fire polishing no matter what glass they were made from. Calibration bar = 10 µm.

PPORT
OCOL 1

OBTAINING OPTIMAL TIP GEOMETRY

Single-Channel Recording Pipets

For single-channel recordings, background noise is minimized by using a glass with favorable electrical properties and by obtaining the highest possible seal resistance. To reliably obtain the highest percentage of high-resistance seals, much longer and higher resistance tips must be used in comparison to those used for whole-cell recording. Thick-walled glass not only improves electrical properties but also seems to promote high-resistance seals. This is more likely due to the small internal diameter of the pipet rather than the thickness of the wall. Figure 6.3.1 shows several forms of single-channel pipet tips before fire polishing; these tips might be expected to give high seal resistances and low background current noise. Glass of the same kind but with different wall thickness can usually be pulled with the same program. Some small changes may be required to optimize the tips but generally usable tips will be formed with no program changes. Schott 8250 with thick walls violates this principle and requires modification of pressure and

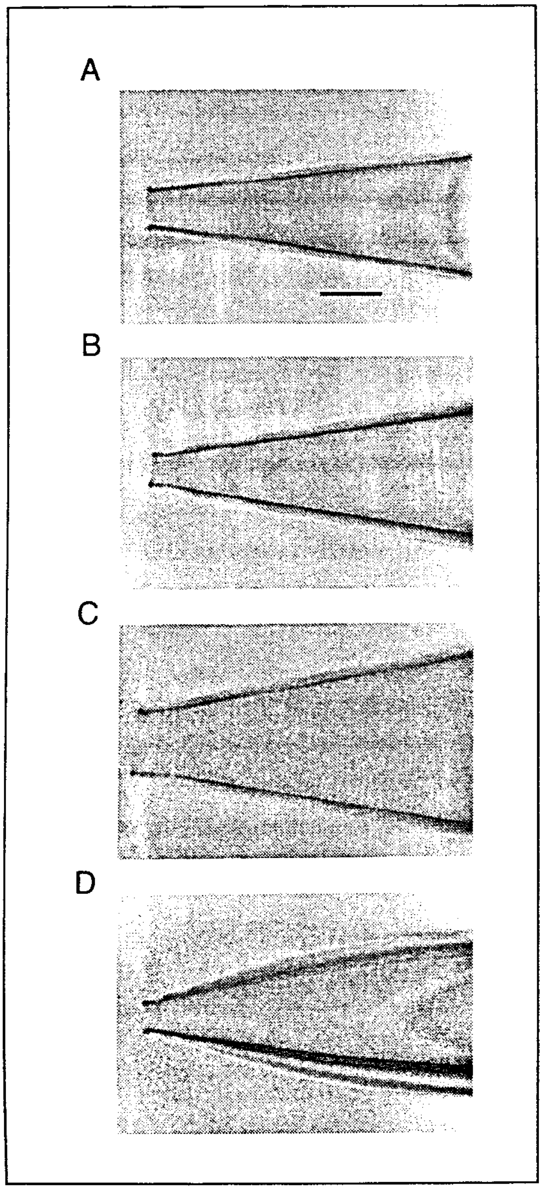

Figure 6.3.2 Tip geometries of patch pipets pulled for whole-cell recording before fire polishing. (**A**) Schott 8330 o.d. = 1.65 mm, i.d. = 1.15 mm, Sutter Program 4, pressure = 600. (**B**) Schott 8250 thin wall o.d. = 1.65 mm, i.d. = 1.30 mm, Sutter Program 3, pressure = 600. (**C**) Schott 8250 thin wall o.d. = 1.65 mm, i.d. = 1.3 mm, Sutter Program 3, pressure = 800. (**D**) Quartz o.d. = 1.50 mm, i.d = 0.75 mm, Sutter Program 1. Quartz geometry is not ideal but is about as good as it is possible to obtain and still hope to obtain gigaohm seals without fire polishing. Calibration bar = 10 μm.

velocity to achieve optimal tip geometries. Recently the authors have obtained the lowest noise results using a single-stage pull which creates a tip that is quite sharp and looks more like the tip of an intracellular electrode than that of a standard patch electrode. A program that works well for these electrodes on the Sutter P-97 puller is as follows:

pressure = 999
heat = ramp value
pull = 50 to 125 (for electrodes of 25 to 75 MΩ)
velocity = 255
time = 255.

Whole-Cell Recording Pipets

To optimize bandwidth, reduce whole-cell noise, and minimize series resistance errors, blunt, low-resistance pipet tips are required. For whole-cell recording, the lowest possible resistance pipet that will still make gigaohm seals is desirable. Using thin-walled glass tubing to pull the pipets will result in the lowest possible resistance. However, perfectly acceptable results can also be achieved with thick-walled tubing in situations where (for whatever reason) relatively large depths of immersion of the pipet are required. In such cases (which should be avoided whenever possible), thick-walled pipets can help to minimize pipet capacitance. Figure 6.3.2 shows examples of optimal whole-cell recording pipet tips before fire polishing.

SUPPOR
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CALIBRATING THE PULLER FILAMENT

This protocol details the recalibration of the puller after a filament has been burned out and replaced with a similar filament.

Materials

Box filament
Fine forceps
Pipet glass
Pipet puller

1. Construct a filament former as shown in Figure 6.3.3.

   This can be turned on a lathe in a machine shop or can be fabricated by gluing together two pieces of glass of the appropriate lengths and diameters.

2. Use a box filament that totally surrounds the glass.

   Sutter does not recommend box filaments for patch pipets because they limit cooling by the gas stream. The authors don't find this to be a problem.

3. Clamp the filament former into position as if a pipet were about to be pulled.

4. Using fine forceps, pull the filament on both ends until it roughly conforms to the outside dimensions of the filament former.

   Some repeated bending and forming of the filament near the former might also be required.

5. Remove the filament former and then clamp a piece of pipet glass in the puller.

   Be sure that the glass is centered in the filament with respect to length so that if pipets are pulled, the two pipets will be of the same length.

6. Run the ramp test (see manufacturer's instruction manual) to find the heat setting at which the glass softens.

   This is the temperature at which the pulling would be done in subsequent pulling programs.

CLEANING THE GLASS

It is usually unnecessary to clean glass which has been redrawn from larger-dimension tubing (as is almost always true for pipet glass), as the new glass is largely pulled from glass that was internal (in the wall) of the larger-diameter glass stock. However, it can be worth testing the glass to see if cleaning is required, particularly for single-channel recording. This can easily be accomplished by pulling a few pipets, filling them, and inserting them into the headstage and holder. If the noise observed with the pipet tip not immersed in the bathing solution is significantly elevated above the appropriate percentage typical of a particular glass (and line frequency or other interference is not the cause), then it is probably worthwhile cleaning and drying the glass prior to pulling more pipets. This may or may not solve the problem, but it is certainly a simple and worthwhile precaution to take prior to seeking other possible causes of elevated noise. Incidentally, in situations like this, a dirty holder (contaminated by filling solution) is often responsible for some or all of the elevated noise. This case can usually be identified by significantly elevated noise when the holder is connected to the headstage without a pipet inserted. Holders can be cleaned and dried by methods generally similar to those in this procedure.

Materials

Pipet glass
Ethanol or methanol
Ultrasonic bath cleaner
100°C oven

1. Immerse pipet glass in a beaker filled with either ethanol or methanol. Sonicate for ~5 min in an ultrasonic bath cleaner.

   Be sure that the sonicator has sufficient fluid so that the level reaches that suggested for the cleaner when the beaker is in position. During sonication be sure that there is a steady stream of bubbles traversing the lumen of each piece of pipet glass, starting at the bottom and rising to the top.

2. After sonication, pour off the ethanol or methanol and resonicate the glass in distilled water. After sonication, pour off the water and place the beaker and electrode glass in a 100°C oven. Bake for 30 min to 1 hr.

3. Allow sufficient time for the glass to cool before using it in an experiment.

   On rare occasions the authors have also observed elevated noise from clean glass in high humidity environments. This can generally be corrected by baking the glass (or pulled but unfilled pipets) in an oven at ~70°C for 1 hr immediately prior to use. This problem has never been thoroughly investigated, since it has only rarely occurred in our laboratories.

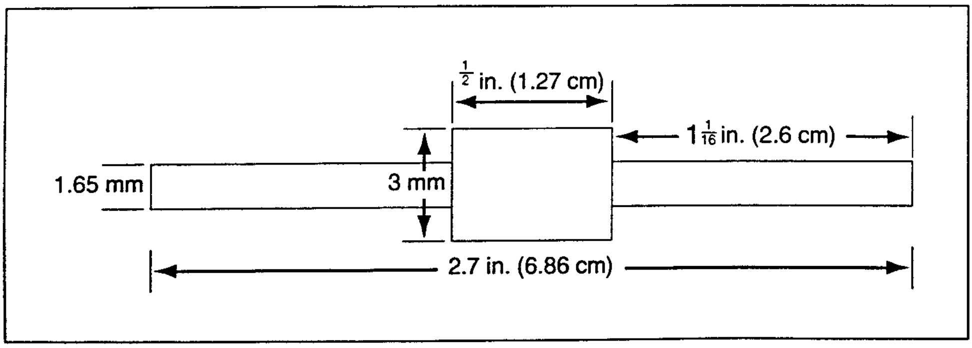

Figure 6.3.3 Drawing of a filament former.

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NOISE CONSIDERATIONS FOR SINGLE-CHANNEL PATCH PIPETS

The geometry and resistance of a patch pipet can also affect its noise performance in several ways. These include **distributed *RC* noise** and **R_e-C_p noise**, both of which have been discussed elsewhere (Levis and Rae, 1992; 1993). Dielectric noise has already been discussed; it is also affected by pipet geometry (especially wall thickness), but is more easily affected by glass type than the other noise sources just mentioned. Finally, pipet geometry can also affect the seal resistance achieved and will thereby affect seal noise. Additional discussions of noise can be found in Background Information.

Distributed *RC* Noise

The authors have used the term "distributed *RC* noise" to describe a component of patch pipet noise arising from the capacitance of the pipet wall and the resistance of its lumen distributed along the portion of the pipet immersed in the bath (Levis and Rae, 1992, 1993). The capacitance of the pipet wall is distributed more or less evenly along the immersed portion of the pipet. However, the resistance is not distributed evenly; the majority of the resistance is located near the tip. Nevertheless, significant resistance remains in the region distal to the tip and this resistance generates thermal noise which, in conjunction with the distributed capacitance of the immersed pipet, produces current noise with a power spectral density that rises as f^2 over the frequency range of interest in patch voltage clamping.

Distributed *RC* noise is minimized by using thick-walled pipets and/or heavy elastomer coatings extending as close as possible to the tip, and by shallow depths of immersion of the pipet into the bathing solution. It is also minimized by reducing pipet resistance, for example by producing relatively blunt-tipped pipets with the diameter increasing rapidly with increased distance back from the tip. However, in many situations this may not be practical, and it may conflict with other desired features of the pipet. Blunt-tipped pipets are also generally more difficult to achieve with high melting temperature glasses such as quartz; these glasses, however, often have other very desirable qualities. Also, high-resistance seals might not be possible routinely with blunt-tipped pipets. Thus, in actual practice it is normally best to minimize this source of noise by using thick-walled glass with heavy elastomer coating and shallow depths of immersion. As described above (see Support Protocol 1), pulling of relatively thick-walled pipets is not much different than pulling pipets with thinner walls made from the same glass. An alternative that can be used with low melting temperature glasses is heat polishing to produce thick walls near the tip in conjunction with heavy elastomer.

R_e-C_p Noise

In single-channel recording, the capacitance, C_p, of the membrane patch itself is usually quite small, in most cases ranging from as little as 1 fF or less (Bendorff, 1995) up to perhaps 0.25 pF (see also Sakmann and Neher, 1983). However, the patch capacitance is in series with the entire resistance of the patch pipet, R_e, which is almost always more than 1 MΩ and can be many tens of MΩ. This resistance has a high thermal voltage noise, and this voltage noise in series with the patch capacitance produces current noise that is called R_e-C_p noise. Up to frequencies of ~$1/(2\pi R_e C_p)$, the power spectral density (in amp^2/Hz) of this noise is given by:

$$S_{ep}{}^2 = 4\pi^2 e_e{}^2 C_p{}^2 f^2$$

where $e_e{}^2 = 4kTR_e$. The root-mean-square (rms) current noise, i_{ep} (in amp rms), arising from R_e in series with C_p is then given by:

$$i_{ep} = (1.33\pi^2 c_3 e_e{}^2 C_p{}^2 B^3)^{1/2}$$

where B is the −3 dB bandwidth in hertz and c_3 is a coefficient that depends on the filter type ($c_3 \cong 1.9$ for an 8-pole Bessel filter). Since tip diameter is a major determinant of both R_e and patch size (and thus C_p), it is not surprising that this noise is determined by the size and geometry of the tip of the pipet. Data presented by Sakmann and Neher (1983) indicates that patch capacitance falls in the range of ~0.01 pF to 0.25 pF for pipets with resistances in the range of 1 to 10 MΩ. There is a large amount of scatter in their data, but, as expected, the trend clearly shows that higher patch capacitances are associated with lower-resistance pipets. It should be noted that the equation for i_{ep} depends linearly on C_p and on $R_e^{1/2}$. Because of this and the expected relationship between pipet resistance and patch capacitance, this source of noise is expected to be minimized by small area patches even if the associated pipet resistance is high (see Rae and Levis, 1994; Levis and Rae, 1992, 1993). For example, a very unfavorable situation in terms of R_e-C_p noise would be $R_e = 2$ MΩ and $C_p = 0.2$ pF; this should produce ~0.18 pA rms noise in a 10-kHz bandwidth. On the other hand for $R_e = 10$ MΩ and $C_p = 0.01$ pF, this noise source would be reduced to ~0.02 pA rms in this same bandwidth. In extreme situations such as those reported by Bendorff (1995), where $R_e \cong 100$ MΩ and $C_p < 1$ fF, this noise source should not exceed ~6 fA rms in a 10-kHz bandwidth. Thus in most situations this noise source is small; it is only expected to become significant when patch area is quite high.

Seal Noise

With zero applied voltage, the power spectral density of the current noise arising from the membrane-glass seal is expected to be given by $4kT\mathrm{Re}\{Y_{sh}\}$, where $\mathrm{Re}\{Y_{sh}\}$ is the real part of the seal admittance. The minimum value of $\mathrm{Re}\{Y_{sh}\}$ is $1/R_{sh}$, where R_{sh} is the DC seal resistance. Excess low-frequency noise is also possible when current is crossing the seal. In addition, it has been suggested that the seal may generate shot noise (Bendorff, 1995). This is possible, although, to the best of our knowledge it has never been demonstrated experimentally. Shot noise occurs when a current, i, flows across a potential barrier; it has a power spectral density given by $2qi$, where q is the electronic charge (1.6×10^{-19}). Since the precise nature of the membrane-glass seal still remains unknown, shot noise is a possibility, but it is not necessary since no potential barrier is expected to be involved (e.g., a resistor does not produce shot noise when current flows through it, but a PN junction does). In any case, it is clear that the amount of noise attributable to the seal (from all possible sources just considered) will be minimized when the seal resistance is as high as possible. The authors have presented evidence that, at least in some patches, seal noise is indistinguishable from the expected thermal current noise of the seal resistance (Rae and Levis, 1992b). In other circumstances, it appears that this noise can clearly exceed this amount.

The authors have observed that higher resistance pipets with smaller tip diameters tend to produce the highest resistance seals. However, seal resistances in the range of 100 to 200 GΩ with pipets with resistances of ~5 MΩ have been observed. In general, factors that determine seal resistance on a patch-to-patch basis are not well known. Nevertheless there appears to be a clear average trend for small-tipped pipets to produce the highest resistance seals. Bendorff (1995) has reported that for patch pipets with resistances of 50 to 90 MΩ (when filled with 200% Tyrode's solution) and tip diameters on the order of 0.2 mm, seal resistances as high as 4 TΩ (4×10^{12} Ω) can be produced. These exceedingly high–resistance seals will almost certainly significantly reduce seal noise. This could become quite important in the measurement of small channel currents at relatively narrow bandwidths (<1 kHz), provided that electronics are used that can take advantage of the potentially low seal noise available. It is not known at present if achieving teraohm seals depends on the type of cells (or glass) used as well as on small tip diameter.

It should also be noted that very small patches will also tend to minimize the probability of the tiny patch membrane containing other charge-translocating processes such as pumps or exchangers. Again the major benefit of this is likely to be at relative low frequencies (narrow bandwidths). Of course, very small patches also will reduce the likelihood of the patch containing the type of channel the investigator is interested in recording from. This restricts the situations in which such very small–tipped pipets can be used.

CONSIDERATIONS FOR WHOLE-CELL PIPETS

SUPPOR
PROTOC

Dynamic Considerations

In the case of whole-cell voltage clamping, the entire pipet resistance, R_e, is in series with the cell membrane capacitance, C_m. Indeed, following seal formation and disruption of the patch, this resistance usually exceeds the resistance of the pipet as measured prior to attachment to the cell. Thus in this section, R_e represents the measured resistance after the whole-cell recording configuration has been achieved. This is normally strongly dominated by the pipet, but it will also include any intrinsic resistance in series with the cell membrane.

This total series resistance (R_e) has a variety of important effects that have been described in several previous publications by these authors and others (e.g., Levis and Rae, 1992; Rae and Levis, 1994; Marty and Neher, 1995). Here only some of the most important of these effects will be summarized. It is well known that when current, I_m, crosses the cell membrane, the series resistance, R_e, causes voltage errors given by R_eI_m. Series resistance compensation circuitry provided in most commercial patch clamp amplifiers can reduce these errors. In addition, any uncompensated series resistance will, in conjunction with C_m, have a filtering effect on the measured current. In the absence of compensation for series resistance, this filtering effect limits the actual bandwidth of measured current to $1/(2\pi R_eC_m)$. The filter is analogous to a simple one-pole RC low-pass filter. As an example, consider that with $R_e = 5$ MΩ and $C_m = 50$ pF, $1/(2\pi R_eC_m) = 640$ Hz. The available bandwidth can be increased by the use of series resistance compensation. If α is defined as the fraction of the series resistance that is compensated ($0 \leq \alpha \leq 1$) and β is defined by $\beta = 1 - \alpha$ (so that β is the fraction of the series resistance that remains uncompensated), then series resistance compensation extends the uppermost usable bandwidth to $1/(2\pi R_eC_m)$. This is an important aspect of whole-cell voltage clamping to always bear in mind. Establishing an external filter bandwidth in excess of the limitations just defined will provide essentially no additional high-frequency information, although it will provide additional noise.

Obviously this problem is minimized by minimizing the product R_eC_m, and by using series resistance compensation. In terms of pipet fabrication, this simply means that tips should be as large as practical and pipets should have blunt tips (i.e., the pipet should increase in diameter quickly as you move back from the tip). The limitation in terms of tip diameter depends on how easily seals can be formed and a whole-cell recording situation achieved in any particular cell size or type. The largest tipped (and hence lowest resistance) pipets that can produce reliable results will clearly minimize the dynamic problems considered here. This is true in both standard and perforated patch whole-cell recording configurations.

Noise Considerations

Whole-cell pipets produce all of the types of noise considered above with the exception of R_e-C_p noise. However, in the whole-cell situation, these sources of noise are generally unimportant in comparison with the far larger amount of noise caused by the thermal voltage noise of R_e in series with the cell capacitance, C_m. This noise source is analogous to R_e-C_p noise, but the capacitance C_m is many times larger than C_p. Because of this, the noise produced in a given bandwidth is also much larger. Also, because the time constant R_eC_m is many times larger than R_e-C_p, the bandwidth limitations just described are also significant considerations in terms of noise (unlike the normal situation for R_e-C_p noise). The power spectral density of the noise arising from R_e in series with C_m is given by:

$$S_{\mathrm{em}}^2 = \frac{4\pi^2 e_e^2 C_m^2 f^2}{1+4\pi^2\beta^2 R_e^2 C_m^2 f^2}$$

where β is once again the uncompensated fraction of the series resistance as defined above (see Dynamic Considerations) and $e_e^2 = 4kTR_e$ as defined previously (see R_e-C_p Noise). Note that for 100% series resistance compensation ($\alpha = 1$, $\beta = 0$) this equation reduces to $S_{\mathrm{em}}^2 = 4\pi^2 e_e^2 C_m^2 f^2$. This is the same form as the expression for the power spectral density of R_e-C_p noise (with C_m substituted for C_p), with noise power increasing as frequency increases in proportion to f^2. When series resistance is not completely compensated ($\beta > 0$), the power spectral density flattens out (to a value of $4kT/\beta^2R_e$) at a frequency of $1/(2\pi R_eC_m)$; the level of the power spectral density will then be maintained until the signal and noise are rolled off by an external filter, or until the bandwidth limit of the electronics is reached.

Under most circumstances, this source of noise dominates whole-cell voltage clamp whenever the bandwidth is more than a few hundred hertz. As an example, consider a situation with R_e = 5 MΩ and C_m = 50 pF. Further consider that series resistance compensation has been set to 80%. This provides an actual bandwidth limitation of 3.2 kHz (as opposed to only 640 Hz without series resistance compensation). In this case, the noise from R_e in series with C_m will be ~2.2 pA rms for a bandwidth of 1 kHz (−3 dB, 8-pole Bessel filter), and will increase to nearly 12 pA rms for a bandwidth of 3 kHz. Both of these values are far higher than the noise of any commercially available patch/whole-cell amplifier at the same bandwidths and are also much higher than the noise attributable to the patch pipet per se as described. Only for very small cells (low C_m) with relatively low values of R_e (which are hard to achieve with small cells) will other sources of noise remain significant (provided, of course, that reasonable precautions are taken). However, even with the lowest values of the product R_eC_m that the authors have experienced which have resulted from small cells ($C_m \cong 6$ pF) with values of R_e as low as 3 to 4 MΩ, this source of noise reaches ~2.5 pA rms in a 5 kHz bandwidth and still dominates total noise at all bandwidths above about a kilohertz or so.

Clearly, minimizing this noise requires that R_e, C_m, or both be minimized. In the case of C_m, this means selecting small cells and this may not always be practical or possible. Furthermore as described by Rae and Levis (1994), assuming a constant value of R_e and that the channels of interest occur in the same density per unit area in cells of different sizes, then signal-to-noise ratio will be independent of cell size. The most practical way to attempt to minimize this noise source is to use the lowest-resistance pipets that will form seals with the cells and allow the whole-cell recording situation to be reliably achieved. As a practical matter, also be aware of the effective bandwidth limitations described (due to C_m and uncompensated series resistance) and avoid setting the external filter bandwidth higher than this limit.

PREPARING PIPET TIPS WITH ELASTOMER COATING

For rendering the external surface of the pipet hydrophobic for all glasses and to improve the electrical quality of all glasses other than quartz, it is necessary to coat the pipet near the tip with an elastomer. There is a fundamental difference in the requirements for coating single-channel and whole-cell recording pipets. For single-channel pipets, noise and capacitance reduction are the main reasons for coating. Therefore, thick coating to as close to the tip as possible is warranted. One anomaly noticed by the authors is that General Electric quartz shows a much reduced tendency for fluid film to creep up the external surface of an immersed pipet tip, making the elastomer coating less important than with other glasses. With whole-cell pipets, the noise from the pipet is usually insignificant in comparison to the noise from the cell capacitance in series with R_e. Coating is required to reduce pipet capacitance and thereby ensure that the capacity transient is small enough to be effectively canceled by the circuitry of the patch clamp amplifier. This usually can be acheived by painting the pipet with a thin elastomer coating only in the region where the final pipet taper begins (~2 to 3 mm back from the tip) so as to limit the outside fluid film to just the pipet tip. This can be done quickly since precise coating to near the tip is unnecessary.

Three elastomers have proven useful. RTV615 (General Electric) and Sylgard 184 (Dow Corning) are comparable in that they have nearly identical dissipation factors and their viscosity and curing properties are about the same. Both elastomers must be mixed thoroughly with a curing agent and will cure at room temperature, so they must be stored frozen (−20°C) in aliquots in 1.5-ml microcentrifuge tubes. When thawing, the tube must be brought to room temperature before opening to ensure that water does not condense in the elastomer, thereby degrading the electrical properties. Both elastomers have the potential of being mixed inadequately, thus producing pockets of incurable elastomer in the mix. These areas have lower viscosity and can easily run into the tip when curing the elastomer on the pipet surface. Because these elastomers will gradually cure at room temperature, their viscosities increase continually with time after removal from the freezer. This property can be useful if exceptionally thick coatings are desired, since thick coatings are more easily achieved if the elastomer is highly viscous at the time of painting. R-6101 (Dow Corning), on the other hand, comes premixed and does not cure at room temperature, so it can be used for several months without a significant viscosity increase. In the authors' laboratory, R-6101 is prepared as follows. The stock bottle is removed from the freezer and allowed to reach room temperature before opening (again, this is to keep water from condensing in the elastomer). The elastomer is then placed in 1-oz. polypropylene jars (Small Parts, Inc., cat. no. PJ-PP22) so that the jars are half-filled. The jars are tightly sealed and placed in an oven at 90° to 95°C for 48 hr, to achieve an optimal viscosity. These times and temperatures can be varied to achieve almost any desired viscosity. If the jar is kept tightly sealed between uses, it can be stored at room temperature for up to one year before the viscosity rises to an unusable level. The authors store the elastomer stock in the freezer, but the individual 1-oz. jars can be kept at room temperature for months or until all of the aliquot is used. In addition, R-6101 has less tendency to crack when it is overheated with a heat gun. It also has a somewhat better dissipation factor than the other two elastomers.

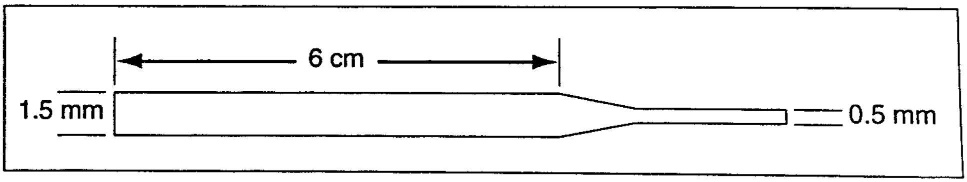

Figure 6.3.4 Effective geometry for wands used to paint elastomer onto patch pipets.

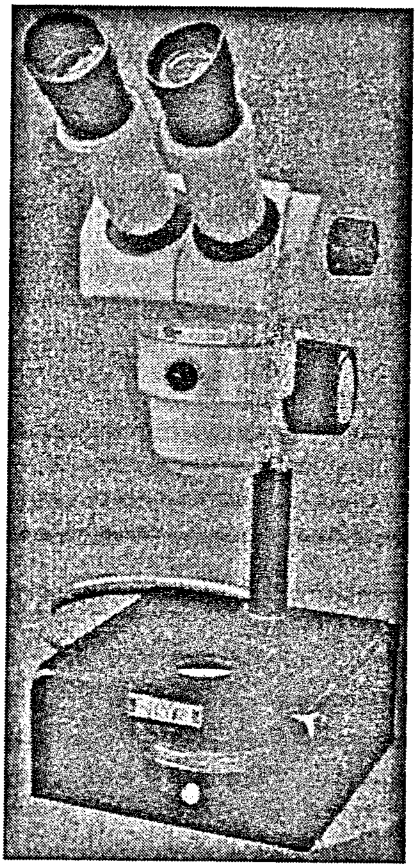

Figure 6.3.5 Dark-field dissecting microscope used for elastomer coating and checking filled pipets for bubbles.

Materials

1- to 2-mm-o.d. × 5- to 7.6-cm (2- to 3-in.) glass tubing or rod (World Precision Instruments)
Pulled pipet (see Basic Protocol 1)
Elastomer: RTV615 (General Electric), R-6101 (Dow Corning), or Sylgard 184 (Dow Corning)
Dissecting microsope, preferably modified for dark-field illumination (Fig. 6.3.5)
Heat gun (e.g., Master Model 10008, Newark Electronics)

1. Using a Bunsen burner and glass tubing or rod of 1- to 2-mm-o.d. × 5- to 7.5-cm (2 to 3 in.) in length, pull the glass into two pieces.

   This is best done by heating the glass in the flame until it softens, removing it from the flame, and then pulling it. If the tip is too long and wispy, simply hold the tip perpendicular to a hard surface and then push the tip against the surface until it breaks off to the desired length and tip diameter. Figure 6.3.4 shows a geometry that works well. Use this pulled glass as a wand to paint the elastomer near the tip of the pipet.

   Alternatively, a pulled pipet can also be used as a painting wand.

2. Remove pulled pipet from micropipet storage jar and examine the tip up to where the taper first begins using a dissecting microscope (Fig. 6.3.5). Use a magnification that allows simultaneous observation of the tip of the pipet and the region up to where the taper first begins.

Dark-field dissecting microscopes are easily constructed using a fiber-optic ring illuminator (obtained from any microscope supply company) at the base of the microscope facing the objective to give good dark-field illumination for a distance of 2 to 6 in. (5 to 15 cm) above the illuminator.

3. From a reservoir of elastomer, dip the glass painting wand into the elastomer and then put a generous blob of elastomer about halfway up the pipet taper.

   An aliquot of elastomer stored in a 1.5-ml microcentrifuge tube is a good reservoir.

4. With the tip of the wand, extend the elastomer to a region above where the pipet taper begins and then to a region as close to the pipet tip as possible. Rotate the pipet about its long axis and be sure to cover all of the pipet glass surface.

   During this operation, be sure that at all times the pipet tip is higher than the rest of the pipet so there is no chance that the elastomer can run (by gravity) into the tip.

5. With the coated tip extending essentially straight up, put the coated tip into the blowing air of a hot heat gun (Fig. 6.3.6).

   Because of the favorable viscosity properties of R-6101, the pipet should be held tip-down when using the heat gun. This results in a much thicker, teardrop-shaped coating over the majority of the shank of the pipet.

6. Continually twirl the pipet so as to uniformly heat the elastomer.

   It should take 5 to 10 sec to cure the elastomer completely. Do not overheat because the elastomer will crack and uncover regions of the electrode especially near its tip.

   Note that when R-6101 is used, cracking and uncovering of the pipet tip usually does not occur.

7. If the coating obtained is not thick enough, simply repeat steps 3 to 6 as many times as desired (Fig. 6.3.7).

   It is most efficient to paint several pipets at a single sitting. Simply turn on the heat gun and leave it on while painting as many pipets as desired.

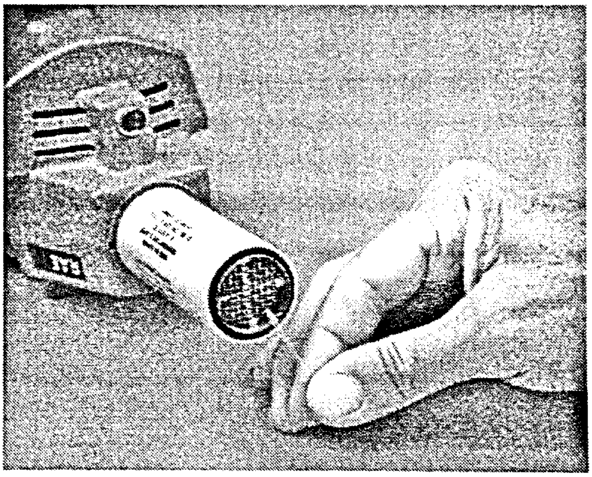

Figure 6.3.6 Elastomer curing with a heat gun. Notice pipet tip points upwards at all times.

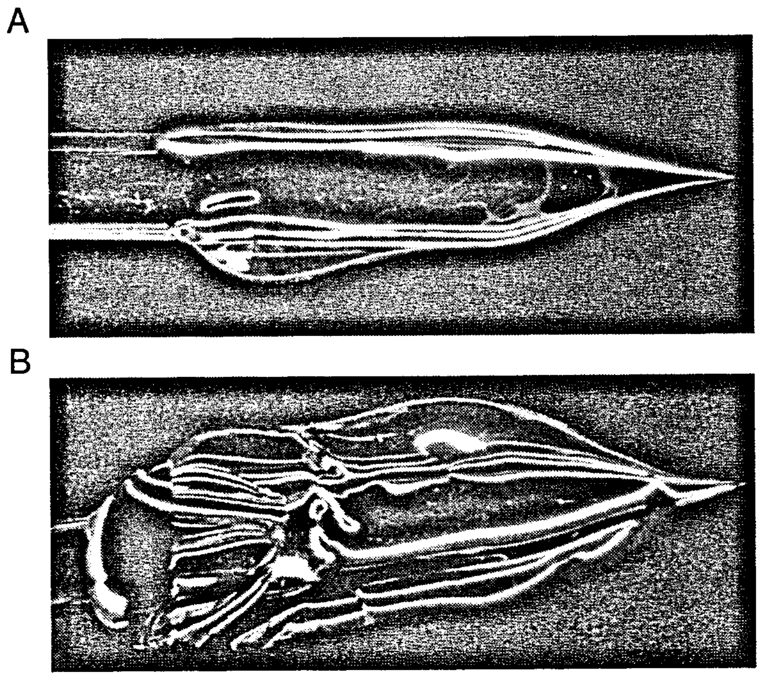

Figure 6.3.7 Elastomer-coated pipets. (**A**) Standard thickness coat: single coating with R-6101. (**B**) Thick coat: triple coating with R-6101. Note effectiveness of dark-field illumination for delineating edges.

CONSIDERATIONS FOR PIPET COATING

Capacity Transients

As discussed in Rae and Levis (1992a), coating of pipets fabricated from most types of glass can reduce the amplitude of both the fast and particularly the slow component of the pipet capacity transient. This is because for any given depth of immersion, elastomer coating will reduce the total capacitance of the pipet and, since the dissipation factors of the elastomers recommended here are less than that of almost all glasses other than quartz, it will particularly reduce the slow component of this transient. Heavier elastomer coatings produce the greatest improvements. Quartz pipets show extremely little slow component in their capacity transients and this is not much changed by the thickness of the elastomer coating; of course; as with other glasses, the total amplitude of the transient (for a given depth of immersion) is reduced in proportion to the reduction of total pipet capacitance.

Noise Considerations

Single-channel patch pipets

Elastomer coating is important to the noise performance of single-channel patch pipets for a variety of reasons. The most commonly used coating is Sylgard 184 (Dow Corning), although a number of alternatives are available. In terms of noise, probably the single most important reason to coat the pipet with an elastomer is to prevent the formation of thin films of solution which usually will otherwise form on the outer surface of an uncoated pipet as it emerges from the bath. Such films have a high distributed resistance which is in series with the distributed capacitance of the pipet wall. In this sense, this source of noise is similar to distributed *RC* noise already discussed (see Support Protocol

4). However, the resistance of the thin film is higher than that of the solution in the lumen of the pipet. In an uncoated pipet, it is expected that the power spectral density of this noise will rise at low-to-moderate frequencies and eventually level out at higher frequencies (in the range of kilohertz to several tens of kilohertz). The authors have estimated that the noise associated with such films is typically in the neighborhood of 100 to 300 fA rms in a 5-kHz bandwidth, but it can be even higher. This is sufficiently large that in many cases it would dominate overall noise. Fortunately, however, coating with an elastomer can essentially eliminate this source of noise. It is assumed that the hydrophobic surface presented by suitable elastomers prevents the formation of these thin films of solution and so eliminates this source of noise.

Elastomer coating can also be quite important to minimizing distributed *RC* noise. This source of noise has already been discussed (see Support Protocol 4), and it should be recalled that it can be minimized by using thick-walled pipets. Such a thick wall can be achieved by either starting with thick-walled glass capillaries or applying a heavy layer of elastomer, or both. This reduces the capacitance of the immersed portion of the pipet wall for any given depth of immersion, and thereby reduces distributed *RC* noise. Sylgard 184, for example, has a dielectric constant of ~2.8, which is less than that of any available glass. This improves its effectiveness in reducing wall capacitance for any particular thickness of coating. Very heavy coats of elastomer can readily be applied (see Basic Protocol 2). In addition, a method has been described which allows this coating to be applied all of the way to the tip of the pipet (Levis and Rae, 1993). However, near the tip of the pipet the relative thickness of the coating is normally significantly reduced due to the tendency of the elastomer to flow away from the tip. Thus, for the lowest noise recordings using thick-walled glass is most effective in minimizing distributed *RC* noise. The authors have generally found that near the pipet tip the o.d./i.d. ratio is less than that of the original glass tubing. However, in some cases this ratio can be more or less preserved during pulling (sharper-tipped pipets seem to more closely preserve the initial o.d./i.d. ratio during pulling). If this ratio is preserved during pulling, then the capacitance of the immersed portion of the pipet is proportional to $1/\ln$ (o.d./i.d.). Thus, increasing the o.d./i.d. ratio from 1.4 to 4 reduces this capacitance by about a factor of 4. Even if the initial o.d./i.d. ratio is not preserved during pulling, the relative improvement obtained by increasing the wall thickness of the tubing is roughly the same. Thicker-walled glass is therefore quite effective in reducing distributed *RC* noise.

For all glasses except quartz, coating with R-6101 (Dow Corning), Sylgard 184 (Dow Corning) or RTV615 (General Electric) is clearly expected to decrease dielectric noise as well. The reason for this is that the dissipation factor of these elastomers (e.g., for Sylgard 184 the dissipation factor is reportedly in the range of 0.0009 to 0.002, although the authors believe that 0.002 is near the correct value) is comparable to or less than that of the best glasses described here other than quartz. The treatment of the noise of two different dielectrics in series was presented in detail in Levis and Rae (1993), so only the basic conclusions of that work will be presented here. For pipets fabricated from glasses other than quartz, coating with Sylgard 184 reduces dielectric noise at any particular depth of immersion, and the heavier the coat of elastomer the greater the reduction in noise. For quartz pipets (with quartz having a dissipation factor 20 or more times less than that of Sylgard), coating with Sylgard 184 actually increases the predicted dielectric noise above that expected for an uncoated pipet. Nevertheless, for most realistic values of the capacitance of the immersed portion of the pipet and of the layer of Sylgard, heavier coats somewhat decrease the total noise of the coated quartz pipet. It is important to realize, however, that the small penalty that may be paid in terms of dielectric noise is more than made up for by the reductions achieved in distributed *RC* noise and particularly the elimination of thin-film noise. Coating with a suitable elastomer remains absolutely essential even for quartz pipets. It is also important to realize that even with a coating of Sylgard, a quartz pipet (of equivalent glass wall thickness) will produce significantly less dielectric noise than any other type of pipet. Finally, if an elastomer can be found with a smaller dissipation factor than that of Sylgard, it could reduce the amount of dielectric noise of quartz pipets further even if the dissipation factor of this elastomer was still greater than that of quartz (Levis and Rae, 1993). R-6101 might be such an elastomer, although in tests to date it has not proven to be significantly better than Sylgard 184. Once again it should be recalled that shallow depths of immersion will always minimize dielectric noise.

The lowest overall pipet noise for single-channel recording will be obtained from relatively small-tipped pipets made from thick-walled tubing of glass with the lowest possible dissipation factor (with quartz easily being the best) and coated with an elastomer such as R-6101; shallow depths of immersion are extremely useful in reducing pipet noise. The use of an elastomer coating remains a vital part of this low-noise strategy.

Whole-cell pipets

As already described, the importance of pipet noise per se is significantly reduced in the whole-cell situation. This is primarily because in most situations the noise of R_e in series with C_m will dominate total noise. Nevertheless, it is normally advisable to use at least a light coating of R-6101, Sylgard 184, or other suitable elastomer on whole-cell pipets to prevent the formation of thin films and their associated noise. In addition, coating reduces the size and complexity of the pipet capacity transient, thereby allowing the pipet capacity

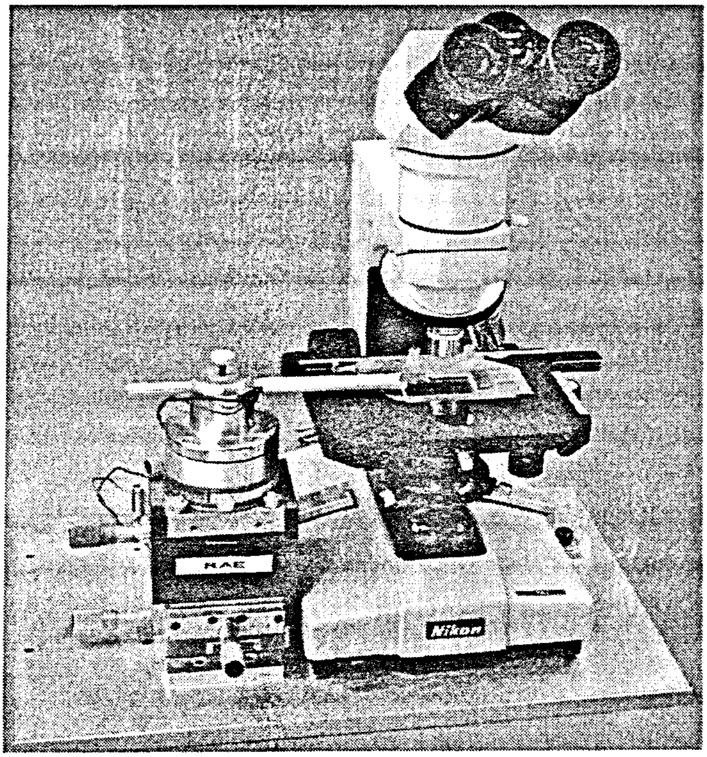

Figure 6.3.8 One possible configuration for a fire polishing setup.

compensation circuitry in most patch clamps to more effectively negate this transient. However, the coating of elastomer need not approach the tip particularly closely and it need not be heavy. With relatively large cells even these moderate precautions may seem unnecessary. However, as a general practice, at least a light coating of elastomer should be used in all cases.

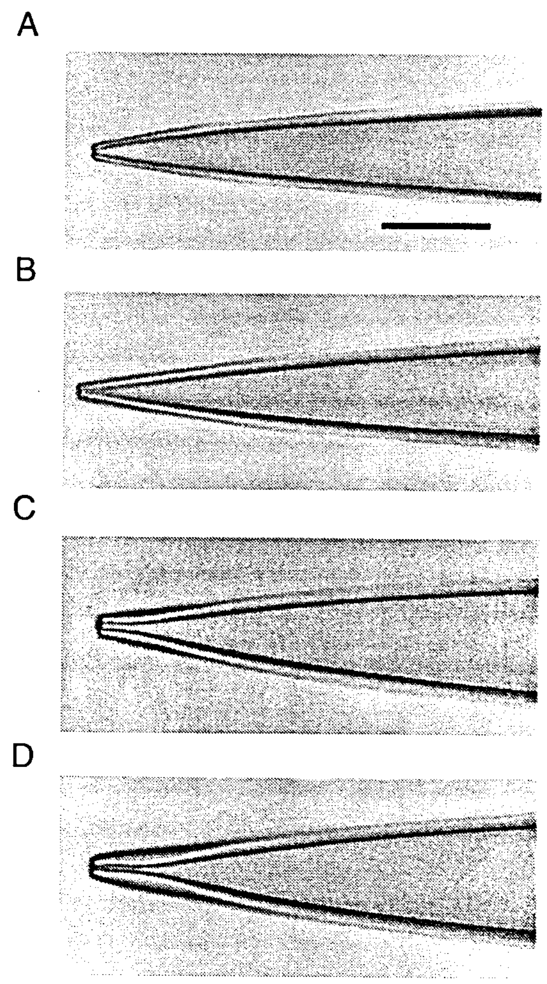

Figure 6.3.9 Pipet tips for single-channel recording following fire polishing. (**A**) Schott 8330 o.d. = 1.65 mm, i.d. = 1.15 mm before fire polishing, Sutter Program 4, pressure = 300. (**B**) Same pipet as (A) after light fire polish. (**C**) Schott 8250 o.d. = 1.65 mm, i.d. = 1.15 mm, Sutter Program 3, pressure = 300 with light fire polish. (**D**) Same pipet as in (C) with additional fire polishing. Note thicker wall near tip and long region of near parallel inner walls. Calibration bar = 10 μm.

FIRE POLISHING THE PIPET

In fire polishing, the tip of the pipet is viewed under a high-power light microscope equipped with long-working-distance objectives. Simultaneously, a glass-coated fine platinum-iridium wire is moved with a micromanipulator into the region of the pipet tip. Current is passed through the wire to heat it sufficiently so that the tip of the pipet can be polished with heat. The current must be adjustable so the temperature of the wire can be varied so as to work with glasses of different melting temperatures or to polish with the wire at different distances from the pipet tip. The objective is to briefly melt the tip so that the glass can flow and make a final tip geometry so that the pipet will not penetrate a cell it is pressed against and will promote seal formation. There are reports of patch clamping some cell types successfully without fire polishing, but for most cells, more frequent and higher-resistance seals are obtained with fire polishing than without it. An exception is quartz or other small-tipped pipets where, in general, fire polishing is really not required for effective seal formation.

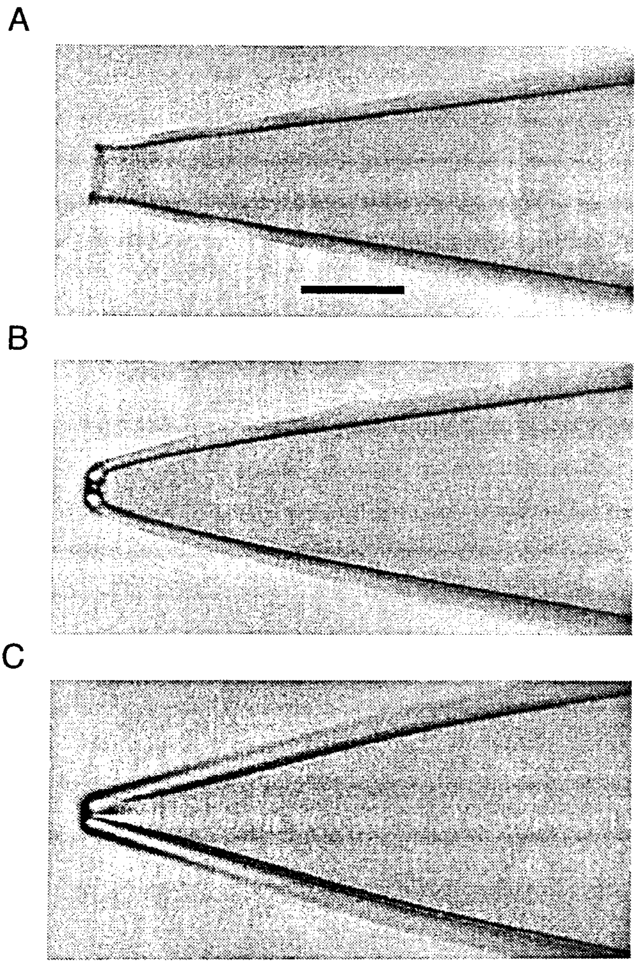

Figure 6.3.10 The effects of fire polishing Schott 8330 (1.65 mm o.d./1.15 mm i.d.) pulled for whole-cell pipets either with the tip near (≈30 microns) or far (≈150 microns) from the fire polishing wire. The unpolished pipet tip, (**A**), narrows and its walls thicken when the tip is closer to the wire, (**B**). When the tip is far away from the wire, its tip simply rounds up (**C**). Calibration bar = 10 µm.

One possible way to configure a fire-polishing apparatus is shown in Figure 6.3.8. With this device, the pipet is placed in a groove in an acrylic plastic microscope slide moved by the X-Y stage micrometer supplied by the manufacturer. The objective is a 100× metallurgical objective with a working distance of 2 to 3 mm; however, most investigators use less expensive 40× objectives with good success. However, for the optimum in visibility, 100× objectives and 15× eyepieces are very useful. This distance is long enough that the heated wire does not melt or otherwise compromise the performance of the objective. The wire is independently movable because it is mounted on the micromanipulator.

Materials

100×, long-working-distance metallurgical objective with 210-mm tube length or infinity corrected (e.g., Nikon, Olympus)
Fire-polishing wire: 0.003-mm platinum-iridium wire (AM Systems)

1. Switch in a 5 to 10× objective and move the tip of the pipet until it is just shy of reaching the center of the optical field.

2. Move the fire-polishing wire, again just shy of the center of the optical field.

3. Switch the 100× objective in place and reposition the pipet tip and the polishing wire.

4a. *For single-channel pipets:* Position the pipet tip and the fire-polishing wire at least 30 µm apart. Adjust the voltage on the heater to the proper level (determined by trial and error) and turn on the current flow. Under direct observation, round and smooth the tip until a small channel can be observed in the region of the tip where the inner glass walls appear about parallel.

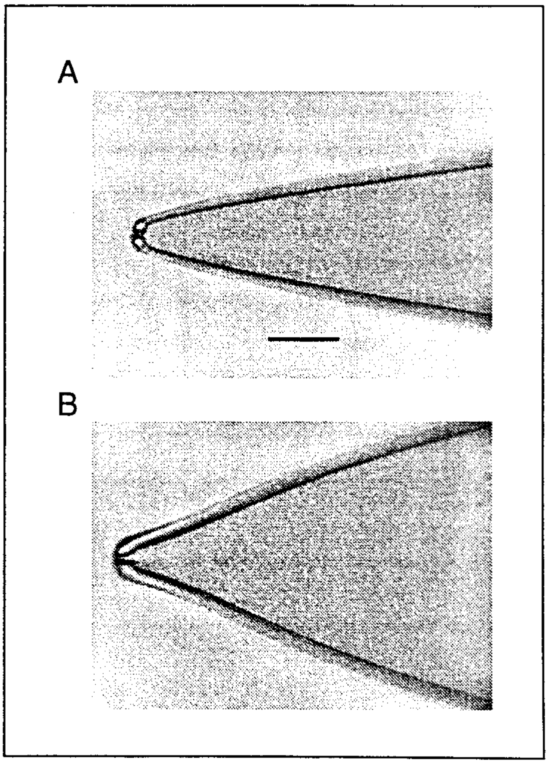

Figure 6.3.11 Pipet tips for whole-cell recording after fire polishing. (**A**) Schott 8330 o.d. = 1.65 mm, i.d. = 1.15 mm, Sutter Program 4, pressure = 600. (**B**) Schott 8250 thin wall o.d. = 1.65 mm, i.d. = 1.30 mm, Sutter Program 3. Pipet in (B) pulled with higher gas pressure (800) than tip in Fig. 6.3.2B (600), therefore the blunter taper. Calibration bar = 10 µm.

The heater wire will spring forward as it expands from the heat and will reduce the distance between the tip and wire.

During this process, it may be necessary to briefly move the tip to within a few microns of the heating wire then quickly move it away again. Repeating this operation several times will give great flexibility in the final geometry of the pipet tip.

Various single-channel pipet tip configurations are shown in Figure 6.3.9.

4b. *For whole-cell pipets:* Move the pipet tip and fire-polishing wire to ~150 µm apart under the high power objective. With the tip and wire now much farther apart, increase the heat until obvious changes in the tip geometry slowly begin to occur.

With the tip and wire at about this separation, the tip should simply round up (see Fig. 6.3.10). This may take up to 30 sec or so. The inner walls of the pipet should not become parallel. Fire polish the minimum amount necessary so as to avoid undesirable increases in tip resistance.

Some tip configurations for whole-cell pipets are shown in Figure 6.3.11.

PPORT OCOL 7

CONSTRUCTING FIRE-POLISHING APPARATUS COMPONENTS

Fire polishing requires a considerable apparatus, some of which cannot be obtained easily from commercial sources. Devices called microforges do exist which would do the entire fire polishing job, but they are expensive, so most investigators have chosen to build at least part of the fire-polishing apparatus. This protocol describes construction of the current source and heating wire.

Constructing a Current Source

A current source for fire polishing is easily made from a Variac (Newark Electronics), a device which varies AC voltage from the line between 0 volts and the full 120 volts or so. This adjustment is too crude, so the Variac output is generally fed through a 20/1 step-down filament transformer (Fig. 6.3.12; Newark Electronics). The resulting voltage range is then 0 to 6 V. Properly constructed fire-polishing heater wires have a resistance

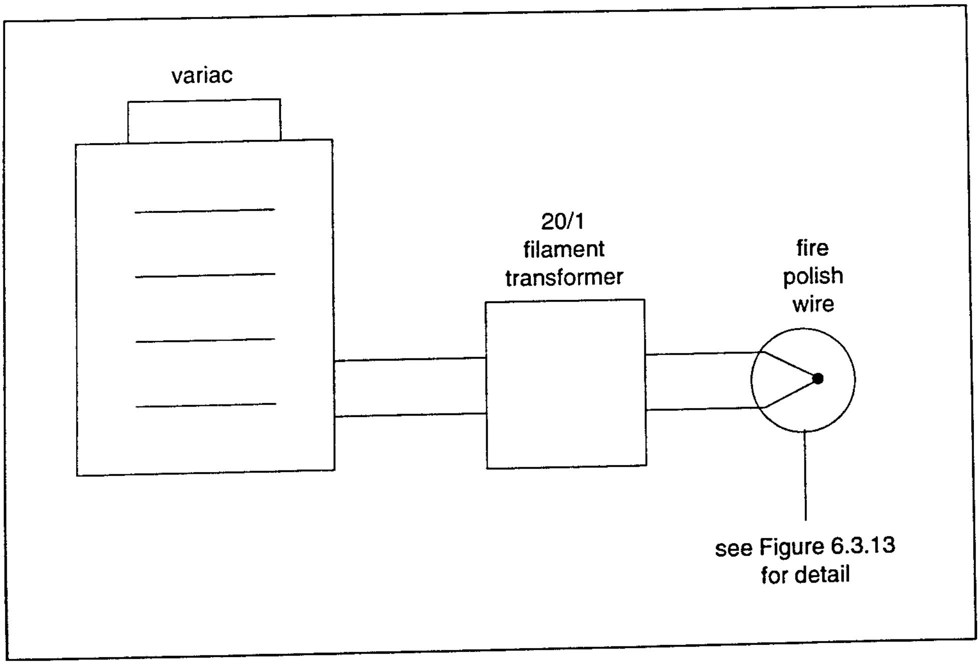

Figure 6.3.12 Block diagram for a fire polishing current source.

of only ~1 Ω. A voltage range of 0.5 to 1.5 V is the usable range for providing the heat required by all glasses the authors have tried. Voltages higher than ~1.5 V will actually destroy the fire-polishing wire and should be avoided.

Constructing a Heater Wire

Two coated solid copper wires of ~22-G are run from the filament transformer output to a cylindrical bar mounted on a micromanipulator. The wires are attached to opposite sides of the bar with electrical tape. Uncoated ends of the wires extend ~1 in. (2.54 cm) past the end of the bar and are bent twice at right angles to produce an end structure like that in the diagram (see Fig. 6.3.13). The portion of the wire that is visible under the microscope is a 0.003-in. (76-μm) diameter platinum-iridium wire (A.M. Systems) that is attached to the end structure of the copper wires. Cut a ~2-in. (5-cm) piece of the platinum-iridium wire and wrap the ends over and over again around the individual pieces of copper wire, leaving ~½ in. (1.3 cm). Bend the remaining wire into a fine hairpin loop (Fig. 6.3.13). Solder the copper and platinum-iridium wires together where the platinum-iridium wire is wound around the copper wire. A proper solder connection will produce <1 Ω of resistance.

Coat the hairpin loop with glass to keep the platinum from sputtering onto the end of the pipet when it is being fire polished. Use the same glass from which pipets are constructed and pull three to four pipets (see Basic Protocol 1). Tape them, one at a time, to a microscope slide on the stage of the microscope. Using a low-power objective and direct observation, move the pipet tip into the vicinity of the hairpin loop. Turn on the heat at near max (~1 V from the current source) and simply jam the pipet tip against the hot wire to melt the tip which will stay on the hairpin loop. Move the pipet away and turn off the heat. Repeat 3 to 4 times until there is enough glass to make a discrete glass bead at the center of the hairpin. Turn on the heat one more time to allow any projections left by the withdrawal of the pipet to melt into the mass of the bead. The heating wire is then ready for use (Fig. 6.3.14).

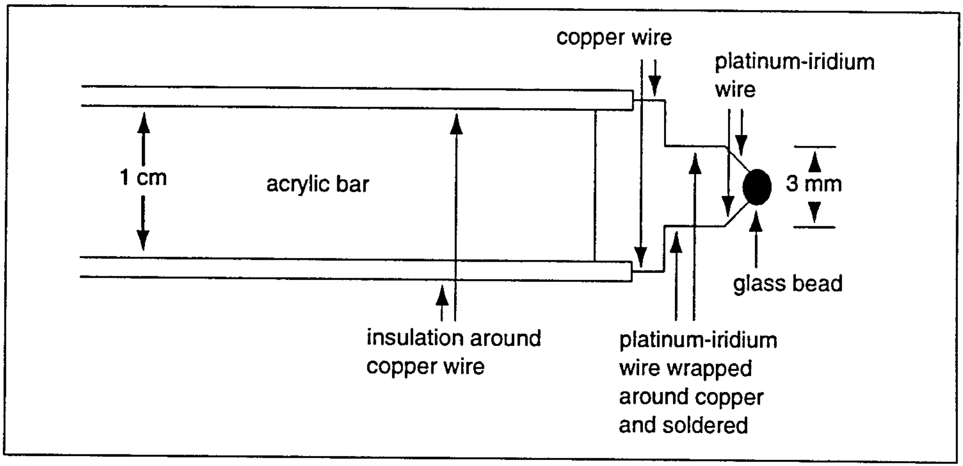

Figure 6.3.13 Drawing of a possible configuration for the fire-polishing wire.

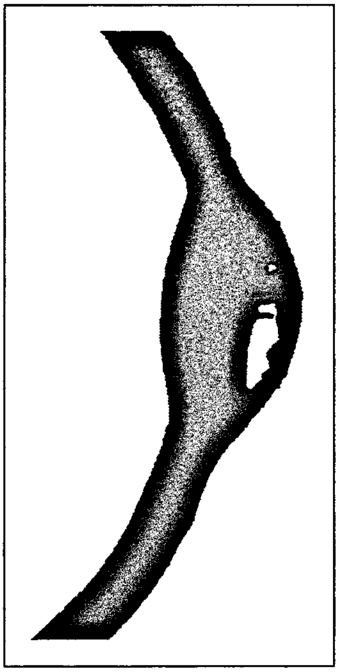

Figure 6.3.14 Glass bead formed on hairpin of fire-polishing wire.

BASIC
TOCOL 4

PIPET FILLING

Pipets must be filled with salt solutions before use. The particular solution used is dictated by the experiment to be performed, so no attempt is made here to describe the composition of filling solutions.

Materials

1-ml tuberculin and 10-ml syringes (Becton Dickinson)
Fire-polished pipet (see Basic Protocol 3)
Suction apparatus
2.0-mm holder (World Precision Instruments)
Needle to fit into the bore of the pipet: e.g., 28-G Microfil (World Precision Instruments), 1.5-in. 22-G Monoject needle, or 1.25-in. 27-G Monoject needle

1. Pull the plunger of a 10-ml syringe to 1 ml.
2. Mount the fire-polished pipet in a suction apparatus as shown in Figure 6.3.15.

   This device is constructed from a pipet holder with the suction line plugged. The upper end is adapted to fit a standard 10-ml syringe. Different holders will require different adaptation schemes. A pipet holder with a male Luer fitting at the top connected to the syringe by Tygon tubing is also convenient.

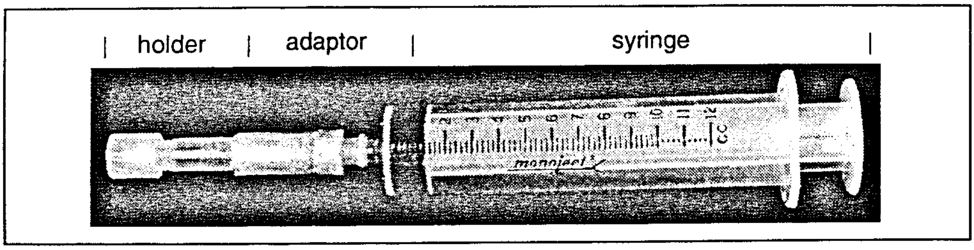

Figure 6.3.15 A syringe and modified pipet holder for drawing suction for pipet filling.

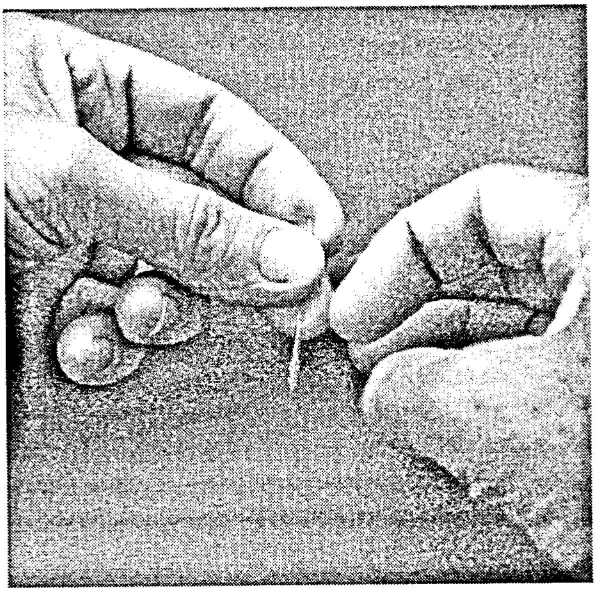

Figure 6.3.16 Removing bubbles from pipet tip by tapping the glass near the pipet shank.

3. After tightening the pipet in place, immerse the tip into a small beaker filled with the proper filling solution. Pull the syringe plunger back to the ~6- to 7-ml mark. Hold for between 5 to 30 sec depending on the tip diameter.

   This should fill the tip of the pipet.

   Many investigators meticulously filter their filling solutions through 0.22- or 0.45-µm syringe filters. Although for special applications this might be required, the authors have not found it necessary when pipets are filled with simple salt solutions.

4. Remove the pipet. Use a 1-ml tuberculin syringe fitted with the proper gauge and length needle to fit into the bore of the pipet to eject a little fluid to clear any solution that might have been in contact with the metal of the needle for a while.

   Alternatively, use a plastic syringe needle (World Precision Instruments).

5. Place the needle into the bore of the pipet from the back until the needle tip bottoms out near the tapered part of the pipet.
6. Inject fluid until the pipet is about half filled and then remove the needle from the pipet.
7. Holding the pipet with the thumb, index, and middle fingers of the left hand, gently flick your right index finger against the pipet where it rests against the index and middle fingers of your left hand (Fig. 6.3.16).

   This should knock out any residual bubbles. Be careful not to tap the glass too hard since the entire piece of glass can break rather easily.

8. Check the pipet tip under the dissecting microscope to verify that all bubbles are gone.

   If bubbles remain, repeat steps 7 and 8 until the tip is bubble free.

MOUNTING AND TESTING THE PIPET SETUP

It is important to mount the pipet in its holder, connect it to the headstage, then quickly test it for excess noise. The pipet holder itself introduces a small amount of noise into the overall measurement. A further increment in noise is associated with insertion of the pipet even before it is immersed in solution. Holder noise arises from the capacitance added by the holder at the headstage input and the dielectric loss associated with this capacitance. It is minimized by using small holders constructed from low-loss materials, such as polycarbonate and Teflon. Holders made from lucite should be avoided for low noise measurements. As with noise sources that are associated with the pipet itself (see Support Protocols 4, 5, and 6 and Background Information), this noise is most important in single-channel recording situations.

Fluid in a pipet holder can make an extensive amount of noise, so it is important not to contaminate the holder interior with pipet-filling solution. Fluid in the pipet can also make thin-film noise in a way analogous to a thin film outside. This protocol describes how to avoid excess noise.

Materials

Bathing solution appropriate for experiment
Pipet
Suction line connected to a syringe needle
Silver/silver chloride reference electrode
Patch clamp apparatus (see *UNIT 6.6*)

Remove excess fluid from holder

1. Dry the outer wall of the pipet with a Kimwipe.
2. Using a suction line connected to a syringe needle of the correct gauge and length to fit into the back of the pipet, suck out the excess fluid leaving the level in the pipet just sufficient to immerse the tip of the internal silver/silver chloride reference electrode when it is inserted.
3. Insert the pipet into the holder and tighten in place.
4. Insert the holder into the patch clamp headstage connector.

Testing the pipet in the setup

5. Position the pipet tip just over the chamber being careful not to actually touch the solution in the chamber.
6. Check the noise on the patch clamp noise meter.

   The noise should not be inappropriately higher than the noise of the headstage alone. Exactly how much noise in excess of the headstage is permissible is a complicated issue and depends on the inherent noise of the patch clamp headstage as well as the type of glass and holder being used. For a resistive headstage with, e.g., 0.13 pA rms noise in a 5-kHz bandwidth, the holder and pipet should increment the noise by not much more than 10% to 20%. For an inherently quieter, cooled, integrating headstage, a 50% increment in noise might be acceptable. This must be empirically determined.

   If the noise increment is too great, it is likely that there is fluid in the holder or that the holder is dirty from some other source. Such a holder can be cleaned by disassembling it and sonicating in ethanol for 3 to 5 min and drying at 70°C for 1 hr or longer (see Support Protocol 3). Be sure the holder has cooled to room temperature before using.

7. If the noise is satisfactory, immerse the pipet tip in the bathing solution and measure its resistance using circuitry inherent to the patch clamp.

   If the resistance is that desired, continue with the experiment. Otherwise, get a new pipet and start over again.

COMMENTARY

Background Information

Pipet noise sources

Several noise sources associated with the pipet have been considered here (see Strategic Planning and Support Protocols 4, 5, and 6). It is important to realize that uncorrelated noise sources add in a sum-of-squares or root-mean-squares (rms) fashion. Thus if the rms value in a particular bandwidth of the individual noise sources considered so far are denoted by:

i_{tf}, thin-film noise
i_{rc}, distributed *RC* noise
i_d, dielectric noise of the pipet
i_{ep}, R_e-C_p noise
i_s, seal noise.

Since these noise sources are all uncorrelated, the total pipet noise, i_p, is given by

$$i_P = \{i_{tf}^2 + i_{rc}^2 + i_d^2 + i_{ep}^2 + i_s^2\}^{1/2}$$

This should be remembered when weighing the importance of the various noise sources to the overall measurement. In addition the noise arising from the holder (i_h) and from the patch clamp headstage amplifier (i_{hs}, including correlated noise from its input voltage noise in series with holder and pipet capacitance) must also be considered when determining total expected noise and when determining the importance of all noise sources. Total rms noise, i_t, is then given by

$$i_t = \{i_{hs}^2 + i_h^2 + i_p^2\}^{1/2}$$

The noise sources associated with the pipet that have been considered above can be summarized as:

Thin-film noise. Thin-film noise results from thin films of solution on the outside and/or inside of the pipet. The power spectral density (PSD) of this noise is expected to rise as f^2 at least into the range of many kilohertz to several tens of kilohertz. This noise can be easily dominate total pipet noise in uncoated pipets. Coating the outside of a pipet with a suitable elastomer will essentially eliminate thin films on the exterior of the pipet and their associated noise. Noise from films on the interior of the pipet and or holder can also be more or less completely eliminated, usually with only minor precautions. Thus, thin-film noise can be a very large problem, but its minimization or effective elimination is not difficult to achieve.

Distributed RC noise. Distributed *RC* noise has a PSD that rises as f^2 over the entire frequency range that is normally of interest to patch clamping (up to 100 kHz or more). Thus, the rms contribution of this noise component increases as bandwidth to the 3/2 power ($B^{3/2}$). Distributed *RC* noise cannot be eliminated, but it can certainly be minimized. The use of glasses with low dielectric constants can reduce pipet capacitance and thus reduce this noise, but the range of dielectric constants of the glasses recommended is only about a factor of 1.5 (3.8 for quartz to 5.1 for Pyrex). This is the only effect of glass type on distributed *RC* noise, and it is therefore apparent that glass type is not very significant to distributed *RC* noise. More important reductions can be achieved by using thick-walled glass capillaries. The authors have used o.d./i.d. ratios up to 4, and ratios as high as 8 have been reported (Bendorff, 1995). Further reductions are also possible by heavily coating the pipet with suitable elastomers. The coating should extend as close as possible to the tip. Using relatively low-resistance pipets with relatively blunt tips can also reduce distributed *RC* noise, but this is not always practical and involves some trade-offs that will be considered. Finally, and of great importance, distributed *RC* noise can be reduced by minimizing the depth of immersion of the pipet in the bath. Although distributed *RC* noise can be a significant source of noise, by taking the described precautions, it is possible to reduce this noise component to ~10 fA rms in a 5-kHz bandwidth.

Dielectric noise. Dielectric noise arises from the nonideal (lossy) characteristics of the capacitance of the pipet and its holder. The contribution of the holder has been briefly described (see Basic Protocol 5). Here we will continue to focus on the pipet. Dielectric noise is characterized by a PSD that rises linearly with frequency. This means that the rms noise contribution of dielectric noise rises linearly with increasing bandwidth, *B*.

Quartz pipets are the best selection for minimizing dielectric noise. However, reasonable results for low-noise recording can be achieved with other glasses by using thick-walled capillaries and heavy coatings of a low-loss elastomer (e.g., R-6101, Sylgard 184). With any type of glass, minimizing the depth of immersion is an important method of reducing dielectric noise. In the case of quartz pipets, coating with available elastomers is actually predicted to somewhat increase dielectric noise of the overall pipet, although the dielectric noise of an elastomer-coated quartz pipet remains much less than that of pipets of any other type of glass of equivalent geometry and depth of immersion. The authors have shown that for quartz pipets with an initial o.d./i.d. ratio of 2 prior to pulling and o.d./i.d. ≅ 1.4 near the tip after pulling, a moderate to heavy coat of R-6101 or Sylgard 184 and an immersion depth of 2 mm, dielectric noise can be held to ~30 to 35 fA rms in a 5-kHz bandwidth (Levis and Rae, 1993); significantly better results can be achieved with greater-wall-thickness quartz and shallower immersion depths. Bendorff (1995, Fig. 5b) presents data that indicates that for a pipet fabricated from Schott 8330 with an o.d./i.d. ratio of 4 preserved during pulling (apparently with a heavy Sylgard coat extending to within ~50 µm of the tip) and an immersion depth of 1 mm, dielectric noise attributable to the pipet is ~70 fA rms in a 5 kHz bandwidth. In the same paper (Bendorff, 1995), the author notes that the "f noise" component (presumably dielectric noise) of this measured data exceeds the theoretical predictions presented elsewhere in that paper; this data seems to contradict some of the conclusions drawn on the basis of those predictions. The net result seems clear: these authors' actual measurements with quartz pipets, when compared with actual measurements of pipets made from Schott 8330, show that with twice the depth of immersion (2 mm for quartz versus 1 mm for Schott 8330) and half or less of the o.d./i.d. ratio (o.d./i.d. = 2 prior to pulling and o.d./i.d. = 1.4 after pulling for quartz; o.d./i.d. = 4 before pulling and o.d./i.d. = 3.85 after pulling for Schott 8330), the dielectric noise of the quartz pipet is ≤50% that of the Schott 8330 pipet. Quartz is clearly the best selection for low dielectric noise.

R_e-C_p noise. R_e-C_p noise is produced by the thermal voltage noise of the pipet resistance in series with the patch capacitance. Like distributed *RC* noise, it has a PSD that rises as f^2 and its rms noise contribution rises as $B^{3/2}$. In general, R_e-C_p noise is minimized by small-tipped pipets that minimize patch area and capacitance. Even though such pipets have a high resistance, R_e, the net effect is to reduce this type of noise. It should be noted that sharp small-tipped patch pipets will produce relatively high distributed *RC* noise. This can be compensated for by using thick-walled glass and heavy elastomer coating and, of course, by minimizing the depth of immersion. R_e-C_p noise is not expected to be a significant component of total pipet noise unless patch area is quite large (which is associated with low-resistance pipets). For very small–tipped pipets, this noise component can be only a few femtoamps rms for bandwidths of 5 to 10 kHz.

Seal noise: Seal noise is probably the least understood of all noise sources involved in the patch clamp technique. The minimum value of seal noise is easily defined as the thermal current noise of the DC seal resistance. However, the noise may well exceed this minimum, particularly when current crosses the seal. Seal noise is basically white, i.e., its PSD does not change with frequency, although some $1/f$ noise may be present at low frequencies. This means that the rms contribution of seal noise to overall noise should vary as $B^{1/2}$ (i.e., the square root of bandwidth). This is quite unlike the noise sources already summarized which vary as B or $B^{3/2}$. Because of this, particularly for very high–resistance seals, the contribution of seal noise is expected to only be of great importance at relatively narrow bandwidths below ~1 kHz. However, it is expected that seal noise (or the low-frequency noise of the amplifier itself) sets the limit on the lowest levels of noise that can be achieved by reducing bandwidth and is therefore of considerable importance when studying very small channel currents at small bandwidths. The minimum amount of noise arising from seals of 1, 10, 100, 1000, and 4000 GΩ in a 1-kHz bandwidth is 126, 40, 13, 4, and 2 fA rms, respectively. In a 5-kHz bandwidth these values increase to 283, 90, 28, 9, and 4.5 fA rms. These values reflect thermal current noise only and are likely to underestimate seal noise in most situations; however, it is clear from this that high-resistance seals are a prerequisite for extremely low-noise measurements to be possible.

For completeness, this list should also include:

Amplifier input voltage noise in series with the pipet capacitance. The entire capacitance added by the pipet and its holder is in series with the input voltage noise of the patch clamp amplifier. This will produce a noise component with a PSD that rises as f^2 (at frequencies above a few hundred hertz). However, since this noise is perfectly correlated with noise arising from all other capacitance associated with the headstage input, the usual rules of rms noise addition do not apply to it (see Levis and Rae, 1992, 1993). However, for commercially available patch clamps, this noise increment will be relatively small in comparison with the other sources of pipet noise already described.

Holder noise. In addition to the noise mechanism just considered, the pipet holder will also add some dielectric noise of its own.

Small holders (capacitance ~0.6 pF) manufactured from polycarbonate and/or teflon add ~15 fA rms noise in a 5-kHz bandwidth; larger holders (capacitance ≅ 1.5 pF) add ~25 fA rms noise in this bandwidth. Holders manufactured from lucite should be avoided for low-noise measurements.

From this summary it should be appreciated that certain pipet characteristics and procedures are uniformly desirable in terms of noise reduction, while others involve some trade-offs. Thus thick-walled glass (i.e., large o.d./i.d. ratios) and shallow depths of immersion are always desirable and will reduce distributed *RC* noise and dielectric noise without adversely affecting any of the other noise sources considered. Similarly, use of low-loss glasses will always be beneficial for low noise by reducing dielectric noise. Coating with a low-loss elastomer is absolutely necessary to eliminate thin-film noise, and it will always help to reduce distributed *RC* noise; in most cases it will also reduce dielectric noise. However, in the case of quartz pipets, the elastomer coat may actually somewhat increase dielectric noise above the value expected for uncoated quartz pipets. Nevertheless, this trade-off is worthwhile and quartz pipets coated with R-6101 or Sylgard 184 still display lower dielectric noise than pipets fabricated from any other type of glass. Finally, the size and geometry of the pipet tip also involve some trade-offs. Very small–tipped pipets tend to be relatively sharp and are likely to exhibit more distributed *RC* noise than other pipets. However, such pipets will minimize R_e-C_p noise and apparently form the highest resistance seals, thereby minimizing seal noise. It is certainly true that increases in distributed *RC* noise can be at least partially compensated for by using thick-walled pipets and heavy elastomer coating. However, it is important to note that in most cases R_e-C_p noise will not dominate total pipet noise and to remember that the noise of a high-resistance seal is likely to be dominant only when relatively narrow bandwidths (typically <1 kHz) are used, as might be the case when studying very small channel currents. Extremely high–resistance seals will be most important at these low bandwidths, and thus the principal benefit of very small–tipped (and often sharp) pipets will be in the study of very small currents and low bandwidths.

In the lowest-noise recording situations that the authors are aware of, total noise from the pipet, its holder, and the seal has been held to ~35 fA rms in a 5-kHz bandwidth. This has only been achieved with quartz pipets. With other types of glass it has been possible to achieve total noise from these sources of as little as 70 to 80 fA rms in this bandwidth. This indicates that under the best of circumstances the noise of the pipet can be held to less than the noise of even the quietest commercially available patch voltage clamps. The authors believe that the techniques and precautions necessary for achieving noise levels this low are not too difficult, although they can be expensive, and that the benefits in terms of increased resolution can be of great importance. Certainly, however, there will be many circumstances in which ultra-low noise is not required. The reader must judge for himself whether the added time and expense are justified in any particular application. In many cases, however, excellent noise performance can be achieved without added expense and with only a few minutes added to the time of an experiment. The habits formed in achieving this level of performance are good ones, and the authors hope that they have made them more accessible by this review.

Critical Parameters and Troubleshooting

The choice and preparation of the glass is a key element in obtaining successful pipets exhibiting the least noise possible. The most important concerns for choosing the glass are discussed in Strategic Planning.

For single-channel pipets, it is generally best to use short pipets with thick walls pulled to quite fine tips. Coat with elastomer as close to the tip as possible and make the coat as thick as possible or practical. Fire polishing the pipet tip is generally required, except for pipets fabricated from quartz and pipets where the tip opening is already small (typically <0.5 to 1 µm) without fire polishing. Fire polish the pipet by bringing its tip close to the heating wire until the interior walls appear parallel to each other and until the walls noticeably thicken near the tip. For whole-cell pipets, it is generally best to use thin-walled glass and pull the tip to be as blunt as possible. Coat with elastomer, but the coating does not need to extend as close to the tip as it does for single-channel patch pipets and it can be relatively thin. Fire polish with the wire farther from the tip so that the tip simply rounds up with almost no wall thickening. Do not allow internal walls to become parallel. Use the largest tip openings that can routinely form seals to the cells being used. For both single-channel and whole-cell recordings it is important to keep the pipet holder clean.

Additional information on critical aspects of pipet fabrication are presented within the individual protocols.

Anticipated Results

An investigator using these protocols should be able to produce patch pipets with a minimum of defects.

Time Considerations

Approximately 1 min is required to pull each pipet pair, so only ~10 min is required to pull enough pipets for an entire day's recording. Coating with elastomer requires <60 sec for each pipet. It takes ~30 sec per pipet for fire polishing, and about 1 min to fill the pipet and check it under a microscope. Within another 2 min the pipet can be mounted in the apparatus and positioned close enough to the cell to begin sealing. If it is necessary to clean the glass before pulling the pipets, an additional hour of setup time is required, but this is not required every day, as a large quantity of glass can be cleaned at one time.

Acknowledgements

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