# Technology of Patch-Clamp Electrodes

## *Richard A. Levis and James L. Rae*

## 1. Introduction

The extracellular patch voltage clamp technique has allowed the currents through single ionic channels to be studied from a wide variety of cells. In its early form (Neher and Sakmann, 1976), the resolution of this technique was limited by the relatively low (~50 MΩ) resistances that isolated the interior of the pipet from the bath. The high resolution that presently can be achieved with the patch-clamp technique originated with the discovery (Neher, 1981) that very high-resistance (tens or even hundreds of GΩ) seals can form between the cell membrane and the tip of a clean pipet when gentle suction is applied to the pipet interior. Although the precise mechanisms involved in this membrane-to-glass seal are still not fully understood, the importance of the GΩ seal is obvious. The high resistance of the seal ensures that almost all of the current from the membrane patch flows into the pipet and to the input of the current-sensitive headstage preamplifier. It also allows the small patch of membrane to be voltage-clamped rapidly and accurately via the pipet, and the mechanical stability of the seal is vital to the whole-cell voltage clamp technique. Of equal importance, the high resistance of the seal greatly reduces the noise it contributes to single-channel measurements. Although the seal can often represent only a small fraction of total patch-clamp noise (particularly as the bandwidth of recording increases), its importance should never be minimized. Without such high

From: *Neuromethods, Vol. 26: Patch-Clamp Applications and Protocols*
Eds: A. Boulton, G. Baker, and W. Walz © 1995 Humana Press Inc.

resistance seals, most of the steady progress to reduce background noise levels would not have been possible.

Of course, the patch pipet is not simply a tool in the formation of GΩ seals. The pipet serves as a fluid bridge that connects the current-sensitive headstage amplifier input to the surface or interior of the cell. The insulating properties (both resistive and, more importantly, capacitive) of the glass that forms the wall of the pipet are also crucial to the ability to measure current originating in the patch and to the background noise levels that can be achieved.

For any patch-clamp measurement, several steps are required to construct a proper glass electrode. First, a glass that has optimal properties is selected. The required properties differ substantially for single-channel recordings and whole-cell current recordings. For single-channel measurements, low noise is the most important electrical parameter, whereas for whole-cell measurements dynamic performance is more important than the contribution of the electrode to the background noise. This is simply because the background noise in a whole-cell recording is dominated by the noise from the electrode resistance (actually, the access resistance) in series with the capacitance of the entire cell. The dynamic bandwidth of a whole-cell recording also depends on the same factors. Therefore, the goal in constructing an electrode for whole-cell recording is simply to make it as blunt and as low in resistance as is compatible with sealing it to the cell. In single-channel recordings, the pipet is a major contributor to the background notice and so requires many subtle considerations to produce an electrode optimal for recording single-channel currents.

As a second step in pipet construction, the electrode glass stock is pulled into a pipet with a tip of optimal geometry. This geometry differs for whole-cell and single-channel recordings. In a third step, the outside wall of the pipet is coated with a hydrophobic elastomer possessing good electrical properties. This procedure is essential for low noise single-channel recordings, but can be done much less carefully for whole-cell recordings. Fourth, the tip is firepolished to round it and clean its surface of any thin film of elastomer coating. This step can also be used to adjust the final tip diameter. Firepolishing promotes seal formation but often is not required. After all these procedures, the electrode can be filled and used.

Several general properties of glasses must be considered when trying to construct optimal electrodes for patch-clamping (*see* Table 1). Thermal properties determine the ease with which desired tip shapes can be produced and they determine how easily the tips can be heat polished. Optical properties often result in a distinct visual endpoint so that tips can be firepolished the same way each time. Electrical properties are important determinants of the noise the glass produces in a recording situation and determine the size and number of components in the capacity transient following a change of potential across the pipet wall. Glasses are complex substances composed of many compounds and most of their properties are determined to a first order by the composition of the glass used. Glass composition may also influence how easily a glass seals to membranes and whether or not the final electrode will contain compounds leached from the glass into the pipet filling solution, which can activate, inhibit, or block channel currents.

## 2. General Properties of Pipet Glass

Before proceeding to the details of electrode fabrication, it is useful to consider in more detail glass properties that are important for patch-clamp pipet construct ion. We will begin with thermal properties. It is important that glasses soften at a temperature that is easily and reliably achieved. This formerly was a stringent constraint, since glasses like aluminosilicates, which melt at a temperature in excess of 900°C, would shorten the lifetime of a puller heating filament so much that their use was unattractive. Quartz, which melts above 1600°C, could not even be pulled in commercially available pullers and so was not used at all. Today, at least one puller exists that will do these jobs easily (P-2000,

Table 1
Glass Properties

| Glass | Loss factor | $\text{Log}_{10}$ volume resistivity | Dielectric constant | Softening temp., C° | Description |
|---|---|---|---|---|---|
| 7940 | .0038 | 11.8 | 3.8 | 1580 | Quartz (fused silica) |
| 1724 | .0066 | 13.8 | 6.6 | 926 | Aluminosilicate |
| 7070 | .25 | 11.2 | 4.1 | — | Low loss borosilicate |
| 8161 | .50 | 12.0 | 8.3 | 604 | High lead |
| Sylgard | .58 | 13.0 | 2.9 | — | #184 Coating cmpd. |
| 7059 | .584 | 13.1 | 5.8 | 844 | Barium-borosilicate |
| 7760 | .79 | 9.4 | 4.5 | 780 | Borosilicate |
| EG-6 | .80 | 9.6 | 7.0 | 625 | High lead |
| 0120 | .80 | 10.1 | 6.7 | 630 | High lead |
| EG-16 | .90 | 11.3 | 9.6 | 580 | High lead |
| 7040 | 1.00 | 9.6 | 4.8 | 700 | Kovar seal borosilicate |
| KG-12 | 1.00 | 9.9 | 6.7 | 632 | High lead |
| 1723 | 1.00 | 13.5 | 6.3 | 910 | Aluminosilicate |
| 0010 | 1.07 | 8.9 | 6.7 | 625 | High lead |
| 7052 | 1.30 | 9.2 | 4.9 | 710 | Kovar seal borosilicate |
| EN-1 | 1.30 | 9.0 | 5.1 | 716 | Kovar seal borosilicate |
| 7720 | 1.30 | 8.8 | 4.7 | 755 | Tungsten seal borosilicate |
| 7056 | 1.50 | 10.2 | 5.7 | 720 | Kovar seal borosilicate |
| 3320 | 1.50 | 8.6 | 4.9 | 780 | Tungsten seal borosilicate |
| 7050 | 1.60 | 8.8 | 4.9 | 705 | Series seal borosilicate |
| KG-33 | 2.20 | 7.9 | 4.6 | 827 | Kimax borosilicate |
| 7740 | 2.60 | 8.1 | 5.1 | 820 | Pyrex borosilicate |
| 1720 | 2.70 | 11.4 | 7.2 | 915 | Aluminosilicate |
| N-51A | 3.70 | 7.2 | 5.9 | 785 | Borosilicate |
| R-6 | 5.10 | 6.6 | 7.3 | 700 | Soda lime |
| 0080 | 6.50 | 6.4 | 7.2 | 695 | Soda lime |

Sutter Instruments, Navato, CA) and so virtually any kind of glass can be used routinely. It is generally true that the lower the melting temperature of the glass, the more easily it can be firepolished. Low-melting-temperature glasses, such as those with high lead content, can be pulled to have tip diameters in excess of 100 µm and still be firepolished to a small enough tip diameter that the pipet can be sealed to a

7–10 µm diameter cell. With such glasses, one has greater control over the final shape of the tip than is possible with higher melting temperature borosilicate glasses. Quartz pipets cannot be firepolished with a usual firepolishing apparatus, although with care they can be firepolished in a temperature-controlled flame.

Electrical properties are most important for providing low noise as well as low amplitude, simple time-course capacity transients. As will be discussed later, it is not possible to achieve low background noise without an elastomer coating the outside of the pipet. In general, glasses with the lowest dissipation factors have minimal dielectric loss and produce the lowest noise. There is a wide variety of glasses to choose from that will produce acceptable single channel recordings, although quartz is clearly the best material to date. Good electrical glasses are also necessary for whole-cell recordings, not because of noise properties, but because they result in the simplest and most voltage- and time-stable capacity transients.

Major chemical constituents in glass are important since they determine the overall properties of the glass and because they are potential candidates to leach from the glass into the pipet filling solution where they can interact with the channels being studied. No glass can be deemed to be chemically inert, since even tiny amounts of materials leached in the vicinity of the channels may produce sufficient local concentrations to interact with channels and other cellular processes. Again, quartz would be expected to have fewer chemical impurities than other glasses, but every kind of glass should be suspected of having an effect on the channels being measured.

## 3. Whole-Cell Pipet Properties: Practical Aspects

### *3.1. Choice of Glass*

Modern computerized pipet pullers are capable of pulling glass with almost any thermal properties (with the exception of quartz) into the proper blunt-tipped geometry that is ideal for whole-cell recording. Therefore, almost any glass can be used to form whole-cell pipets. Nevertheless, we feel that some types of glass should usually be avoided, whereas others have some particularly useful properties for this application.

Soda lime glasses, such as Kimble R-6 and Corning 0080, generally should not be used because of their high dielectric loss. When a voltage step is applied across a patch pipet fabricated from one of these glasses, there will be a large slow component in the resulting capacity transient (Rae and Levis, 1992a). For a 2-mm depth of immersion with a moderate coating of Sylgard 184 to within ~200 µm of the tip, we have found following a 200 mV voltage stop that is a slow component for a soda lime pipet can be as large as 50 pA 1 ms after the beginning of the step. The slow tail of capacity current can still be as much as 10 pA 10 ms after the step and may require as much as 200 ms to decay to below 1 pA. The time-course of this slow tail is not exponential, but more closely approaches a logarithmic function of time. In addition, we have observed that for soda lime pipets the magnitude of the slow component of capacity current is not always constant during a series of pulses that occur at rates faster than about 1–2/s. Instead, the magnitude of this component is sometimes observed to decrease with successive pulses. Because of these characteristics, these capacitive currents can possibly be mistaken for whole-cell currents. Heavy Sylgard coating can reduce the amplitude of the slow component of capacity current for soda lime glasses, but it is generally better (and certainly more convenient) simply to use glasses with lower loss factors (*see* Rae and Levis, 1992a, for further discussion).

High-lead glasses, such as 8161, EG-6, EG-16, 0010, 0120, and KG-12, possess much lower loss factors than soda lime glasses and are particularly useful because of their low melting point. This property allows the construction of initially very large-tipped pipets that subsequently can be firepolished to blunt bullet-shaped tips offering the lowest possible access resistance. This, of course, minimizes series resistance. In addition, pipets of this shape also draw in the largest surface area patch of membrane when suction is applied. This is useful in perforated patch recordings, since the larger area of membrane available for partitioning by amphotericin or nystatin results in the maximum incorporation of perforation channels and thus the lowest access resistance. KG-12 (Friedrich and Dimmock, Millville, NJ) is a good choice for glasses of this class, since it seals well, has good electrical properties, and is readily available.

Pipets for whole-cell recording can be thin-walled by comparison to those for single-channel recording. In whole-cell measurements, other sources of noise far outweigh the contribution from the pipet *per se* (*see* Section 5.8). In terms of total background noise, the major consideration in pipet fabrication is simply achieving the lowest possible resistance. Glass with an OD/ID ratio of 1.2–1.4 will have lower resistance for a given outside tip diameter than will thicker-walled glass, and is therefore useful for whole-cell recording. Some precautions are necessary, however, since if the walls become too thin the pipet will more easily penetrate the cell during the attempt to form a seal.

Other glasses that have been successfully used by many laboratories for whole-cell recording include Pyrex (Corning [Corning, NY] #7740), Kimble's Kimax, and Corning 7052. Although we usually prefer the high-lead glasses described earlier, these glasses have produced perfectly acceptable results. Note, however, that Corning no longer makes 7052 and so existing supplies will be depleted within a few years.

### 3.2. Pulling Whole-Cell Electrodes

This can be done on any commercially available electrode puller. Here one simply strives for as blunt a taper and as large a tip diameter as is compatible with sealing of the electrode to the cell.

### 3.3. Elastomer Coating Whole-Cell Electrodes

Elastomer coating of electrodes reduces electrode noise in single-channel recordings. In whole-cell recordings, the noise associated with electrode glass is usually insignificant in comparison to other noise sources and so elastomer coating is not required for noise reduction. Elastomer coating also reduces electrode capacitance. Commercial patch-clamp amplifiers have the ability to compensate about 10 pF of electrode capacitance. For pipets made from glasses with high dielectric constants (e.g., soda lime and high-lead glasses) immersed deeply into a tissue bathing solution, the electrode (and holder) capacitance may exceed the compensation range of the electronics. Elastomer coating will help to keep the total electrode capacitance within the compensation range. For whole-cell recordings, it is not usually necessary to paint the elastomer close to the tip. Coating that extends from the top of the shank to 1 mm from the tip is sufficient for whole-cell recordings. Many investigators do not use elastomer coating for whole-cell recordings.

### 3.4. Firepolishing Whole-Cell Electrodes

Finally, to promote GΩ seals and to reduce the possibility of tip penetration into the cell during seal formation, electrode tips should be firepolished. In some cells, firepolishing has proven unnecessary, but we have found that sealing is generally promoted by firepolishing the electrode tip, particularly for cells where seal formation is difficult. Whole-cell and single-channel electrodes are firepolished with the same basic apparatus. Firepolishing can be done either using an upright or an inverted microscope. In fact, many investigators have chosen to coat their pipets and firepolish them using an inverted microscope with a 40× or so long working distance objective.

Another very useful approach is to utilize a standard upright microscope converted to the 210-mm tube length that is standard for metallurgical microscopes. Several microscope companies, but particularly Nikon (Garden City, NY), make extra long working distance and super long working distance high magnification metallurgical objectives. Most noteworthy are the 100× ELWD or 100× SLWD that have 1- mm and 2-mm working distances, respectively. With these objectives and 15× eyepieces and with the electrode mounted on a slide held in the mechanical stage of the microscope, it is possible to move the electrode tip into the optical field and directly visualize the electrode tip at 1500× magnification. At such high magnifications, it is possible to firepolish the tip to a very distinct optical endpoint under direct visualization. This approach ensures very repeatable results from one electrode to the next. The firepolishing itself is accomplished by connecting to a micromanipulator a rod of inert material to which has been fastened a short loop of platinum iridium wire. The ends of this wire must be soldered to two other pieces of wire that can be connected to a voltage or current source to allow current to be passed through the platinum wire. The platinum loop generally is bent into a very fine hairpin so that it can be brought to within a few millimeters of the electrode tip under direct observation. Because of early reports that platinum can be sputtered from the wire onto the electrode tip and prevent sealing, the platinum wire is generally coated with a glass like Pyrex (Corning #7740) or Corning #7052 to prevent such sputtering. This is done by overheating the platinum wire and pushing against it a piece of electrode glass that has been pulled into an electrode tip. At high temperatures, the glass melts and flows over the platinum wire ends up thoroughly coating it and forming a distinct bead of glass. If the elastomer has been coated too near the tip, firepolishing causes the tip to droop downward at the juncture where the coating ends. If one desires to paint elastomer extremely close to the tip, it may be necessary to do the majority of the firepolishing before coating and then firepolish lightly again afterward. As a general rule, firepolishing with the electrode tip close to the heating wire at low temperature produces a tip whose inner walls are parallel and relatively close together. With a hotter heating element and the tip farther away, the tip tends to round more and end up quite blunt.

## 4. Patch Electrode Fabrication for Single-Channel Recording

### 4.1. Choice of Glass

A limited number of glasses are available for single-channel patch-clamping. Perhaps the most important feature to consider is the amount of noise in the recording that is owing to the pipet itself. This subject is sufficiently important that we include an entire section dealing with noise sources in pipets in the hope that readers will be able to use the principles to make optimal pipets for their own recording situation. There is no longer any question, however, that quartz is the best glass if noise performance is important. Quartz itself is quite expensive and requires an expensive laser-based puller, and so probably is not the glass for routine studies. Therefore, we consider other glasses here as well. Garner Glass (Claremont, CA) has been particularly helpful in the development of specialty glasses for patch-clamping, although they are no longer able to provide any of the high-lead glasses we find so useful. Any glass tubing selected for the fabrication of patch electrodes should have walls of substantial thickness. Wall thickness results in decreased electrical noise and increased bluntness at the tip, which prevents penetrating the cell during seal formation. Glass tubing with an OD/ID of 2.0–3.0 is easily obtainable and is expected to yield the lowest background noise levels. Generally, the outside diameter chosen is 1.5–1.7 mm. For single-channel recordings, only the glasses with the best electrical properties should be used if optimal noise performance is desired. Corning glasses #8161 and #7760 are particularly good in this regard, but again Corning no longer makes them and the existing supplies are extremely limited. Corning #7052 is also quite acceptable but also will not be available for much longer. Sadly, most of the options for particularly low-noise glasses are running out, and so quartz is expected to become increasingly more attractive even given its cost. Readily available glasses, like Corning 7740 or Kimble's Kimax, are not particularly quiet glasses. High-lead glasses like Kimble's KG-12 give better signal-to-noise ratios than the Pyrex-type glasses, but are substantially worse than the best glasses mentioned earlier.

In our experience, it is usually unnecessary to clean electrode glasses prior to pulling. On occasion, however, normally quiet pipet glasses are found to be noisy in use, and it is imperative to clean the glass for best noise performance. Sonicating the glass in 100% ethanol or methanol in an ultrasonic cleaner is effective for this purpose. Following any cleaning procedure, it is a good idea to place the glass in an oven at around 200°C for 10–30 min to achieve complete drying. Heat treatment of this sort has also proven necessary if low-noise recordings are required in environments where the humidity is exceptionally high.

### 4.2. Pulling Single-Channel Electrodes

Single-channel pipets made from glasses other than quartz can be pulled on any commercially available patch electrode puller. Here the tips can be less blunt and higher in resistance. The electrode resistance in series with the patch capacitance is a potential noise source (*see* Section 5.5). However, as will be seen, this source of noise actually may be minimized by using high-resistance pipets insofar as such high resistance correlates with a small patch area. In addition, sharper tips taper, often leading to higher resistance seals to the membrane. Thus, for best noise performance for single-channel recording it is better not to use the blunt electrode tips that are good for whole-cell situations.

### 4.3. Coating Single-Channel Pipets with Elastomers

For the lowest noise recordings, electrodes must be coated with a hydrophobic elastomer to within 100 µm or less of their tip. The closer it can be painted to the tip the better. This coating prevents bathing solution from forming a thin fluid film along the outer surface of the electrode. This thin film of bathing solution would be a substantial noise source. A commonly used compound is Sylgard #184 (Dow Corning, Midland, MI). Sylgard also has exceptional electrical properties (*see* Table 1) and so improves the electrical properties of most glasses when a thick coat covers the glass surface. Sylgard, meticulously mixed, can be stored at –20°C in small capped centrifuge tubes. The thorough mixing is required to prevent pockets of the compound not adequately exposed to polymerizer. This unpolymerized elastomer can flow to the electrode tip (even against gravity) and render the tips difficult to seal. At freezer temperatures, the mixed Sylgard can be stored for several weeks. A tube of this freezer-stored Sylgard, when brought to room temperature for use in painting electrodes, will last for several hours before it begins to polymerize. Care must be taken not to open the tube until the contents have reached room temperature to prevent water condensation. Condensed water can degrade the electrical properties of the elastomer and increase noise. The Sylgard is applied to the electrode tip with a small utensil, such as a piece of capillary tubing pulled to a reasonably fine tip in a flame. Sylgard is applied using dissecting microscopes at magnifications of 10–30×. It is useful, but not required, to modify the dissecting microscope to work in a dark field. This can be done inexpensively with a fiberoptic ring illuminator connected to a fiberoptic light source. The ring illuminator is placed under the stage of the microscope. Three to four inches above the ring light, dark-field illumination is achieved and the walls of the electrode glass show up as bright lines of light against a dark background. Both the Sylgard coat and the tip of the electrode are easily seen with this dark-field illumination. The Sylgard must be directed away from the tip by gravity at all times during the painting procedure or the Sylgard may flow over the tip to make firepolishing and/or sealing impossible. The Sylgard can be cured by holding the tip for 5–10 s in the hot air stream emanating from a standard heat gun like those used in electronics to heat shrink tubing. Again, the Sylgard must be gravitationally directed away from the tip during this curing process.

Although Sylgard is the most commonly used elastomer, there are a number of other elastomers available that are as good as Sylgard in most respects and better in others.

RTV615A from General Electric has properties nearly identical to Sylgard and can be used in exactly the same way as is Sylgard. Dow Corning Medical Silastic MDX-4 has dielectric properties slightly better than Sylgard #184 but polymerizes more rapidly at freezer temperatures. To date, it has not offered any obvious improvement in noise on a day-to-day basis, but several of the lowest noise measurements done with quartz electrodes utilized this elastomer. It is considerably more expensive than Sylgard #184. Dow Corning #R-6101 is another excellent elastomer, which costs more to buy, but probably not to use, than Sylgard. R-6101 is useful because it does not polymerize appreciably at room temperature and so can be used for up to 2–3 mo without freezing. Its noise properties are as good (should be a little better) as Sylgard #184 and it does result in low noise when used with quartz or some other very good electrode glass. Teflon AF (Dupont, Wilmington, DE) is a Teflon-based coating material with dielectric properties claimed to better than Sylgard. Its solvent must be obtained from 3-M and both the compound and its solvent are expensive. However, it offers some potential to improve electrode noise when procedures are worked out to use it optimally.

### 4.4. Firepolishing Single-Channel Pipets

The same principles apply here as in the firepolishing of whole-cell electrodes. The same apparatus is used for both. In general, patch electrodes are firepolished with the tip close to the heating filament with the goal of thickening the glass near the tip in addition to rounding it. For high resistance seals, it may be useful to firepolish so that the internal walls of the tip become parallel for several microns. This mode of firepolishing will increase the tip resistance a few MΩ but will often result in lower noise because of higher resistance seals (*see also* Section 5.5).

### 4.5. Fabrication Methods Specific to Quartz

Quartz softens at about 1600°C, and so no platinum or nichrome wire-based heat source will melt it because both of these materials disintegrate long before 1600°C is reached. Quartz can be pulled in a flame, but the tip geometry is unreliable with such fabrication techniques. The new laser-based P-2000 electrode puller from Sutter Instruments generates enough heat to pull quartz fairly easily. It begins to have trouble when the glass OD exceeds 1.5 mm. It has no difficulty pulling quartz tubing with an OD/ID = 3 so long as the OD does not exceed 1.5 mm. Since the major reason to use quartz patch pipets is for the reduction of single-channel background noise currents, it is best to use quartz with as thick a wall as possible. A 1.5-mm OD with 0.5 mm ID produces about the smallest bore that is practical. Even at 0.5 mm ID, there is some difficulty with the internal Ag-AgCl electrode since it must be made of such flimsy silver wire that it is often damaged (bent) or denuded of silver chloride as the electrode is placed into the small bore. IDs of 0.6–0.75 mm make the pipets much easier to use.

Quartz cannot be firepolished easily with any presently available commercial apparatus. Those that firepolish other glasses, including aluminosilicate, do not generate enough heat to firepolish quartz. It is possible to firepolish it in a carefully controlled Bunsen burner, but that approach is sufficiently unreliable that it is best to try to pull tips whose geometry is good enough to allow sealing without firepolishing. That places an additional constraint on the puller, since most other glass pullers need only to produce electrode tips that are approximately correct since the final tip geometry can be customized while firepolishing. With quartz, the tips must be good enough for use immediately after pulling.

Because of the noise produced by a thin film of bathing solution creeping up the outer surface of an electrode, quartz must be elastomer-coated like any other glass. This bathing solution film is such a large noise source that if an elastomer coating is not used to reduce it, there is absolutely no reason to use quartz electrodes for patch-clamping. It will not perform appreciably better than poor glasses if this noise source is not eliminated or minimized. Because quartz must be elastomer coated, it must also be subjected to the heat polisher. Although the polisher cannot smooth or round the quartz tip as it does with other glasses, it can burn off any residual elastomer and so should be used with quartz electrodes just before filling.

### *4.6. Low-Noise Recording*

Low-noise recording requires meticulous attention to detail. Even with an electrode optimally pulled, coated, and firepolished, there are still many ways in which excess noise can creep in. It is important that the electrodes be filled only to just above the shank. Fluid in the back of the electrode can cause internal noise-generating films and allow fluid into the holder. It is important for low-noise recordings that a suction line with a syringe needle the correct size to fit into the bore of the pipet be maintained near the experimental setup. This suction line can be used to vacuum fluid from the pipet and ensure none gets into the holder or coats the majority of the back of the electrode. Alternatively, silicone fluid or mineral oil can be used to fill the electrode for a short distance in back of its filling solution. These "oils" are somewhat messy and not really required if a proper suction line is used. The internal electrode should be adjusted in length until its tip just comfortably is immersed in the filling solution. In general, the shorter the length of the internal electrode (and of the pipet), the lower the noise will be. Therefore, it is best to use the shortest possible holder and electrode that is practical.

During experiments where low noise is required, it is best to test the noise at intermediate stages. Most modern patch-clamp amplifiers have a root mean square noise meter that can be checked to determine the noise levels at any time. This meter should be checked immediately after inserting the electrode into the holder and placing the electrode tip over the bath but before actually immersing the tip in the bath. Poorly filled electrodes, fluid in the holder, a generally dirty holder, and pickup from the environment will show up as elevated noise. What the actual level of the noise will be depends on the noise of your patch-clamp, the kind of holder and electrode glass you are using, and on how well you have shielded against pickup of electrical interference. Specific examples appear in Levis and Rae (1993). As a general rule, however, total noise in this situation should not be more than ~10–20% above that of the open circuit headstage. If you see excess noise, you can remove the electrode, dry the internal electrode, and then test the noise with only the headstage and holder placed above the bath. If this is elevated above what is normal for your setup, either your holder is dirty or you are experiencing pickup from the environment. Environmental pickup often can be seen as noise spikes at discrete frequencies, whereas a dirty holder contributes noise across a broad range of frequencies. You can try to dry the holder by blowing dry, clean air through it, but it is possible that you will have to clean the holder before the noise will go down. This can be done by disassembling it, sonicating it in ethanol, and drying it for several hours in an oven at 60–70°C. Because of the time involved in cleaning the holder, it is wise to have two or more holders available when attempting very low-noise recordings.

The noise of your electronics, holder, electrode glass, and elastomer can be determined by making a thin pad of Sylgard and placing it in the bottom of your chamber. Then seal your electrode to it much as you would sealing to a cell. No suction, however, is required to make the seal. Simply push the tip against the Sylgard and a seal forms. The seal should be 200 GΩ or more if you have done it correctly. Under these circumstances, the seal noise is essentially negligible and you are able to quantify the remaining composite noise sources. This noise will depend on how deep the bathing solution is: The deeper the bathing solution, the greater the noise. For most purposes, the bath depth need not be more than 1–3 mm. This simple procedure will let you know what is routinely possible with your setup and give you a baseline for comparing the noise you actually get in experiments. A good seal to a cell will often produce noise that is about the same as the noise you get sealed to Sylgard.

Note, however, that as soon as the electrode tip is placed in the bath, the noise will be enormous since you are now measuring at best the thermal noise of a 1–10 MΩ resistance tip. The readings on the noise meter will not be meaningful until you have obtained a GΩ seal. If the seal resistance is <20 GΩ, the majority of the noise will be owing to the seal and really low-noise recordings cannot be achieved.

## 5. Noise Properties of Patch Pipets

### *5.1. Noise Contribution of the Pipet*

The earliest patch pipets were fabricated from "soft" soda lime glasses. Such glasses were easy to pull and heat polish to any desired tip geometry, primarily because they soften at relatively low temperatures. Unfortunately, such pipets introduced relatively large amounts of noise into patch-clamp measurements. It was soon found that "hard" borosilicate glasses produced less noise, but, owing to their softening at higher temperatures, were somewhat more difficult to pull and heat polish. Probably as a result of these early findings, it has sometimes been assumed that "hard" high-melting-temperature glasses necessarily have better electrical properties than "soft" low-melting-temperature glasses. However, there is no obligatory relationship between the thermal and electrical properties of glass. For example, several low-melting-temperature high-lead glasses (e.g., 8161, EG-6) have been shown to produce less noise than a variety of high-melting-temperature borosilicate and aluminosilicate glasses (e.g., 7740, 1720). The reason for these findings becomes clear when the electrical properties of the glasses are considered.

The electrical properties of glass that are important to its noise performance are its dielectric constant and its dissipation factor; the bulk resistivity of a glass might also be important, but is usually sufficiently high to be ignored. The dielectric constant of a substance is the ratio of its permittivity to the permittivity of a vacuum. Thus, for pipets of equivalent geometry and depth of immersion, the higher the dielectric constant of the glass, the higher the pipet capacitance. The dielectric constants for glasses commonly used for patch pipet fabrication range from 3.8 for quartz to more than 9 for some high-lead glasses. The dielectric constant of borosilicates is typically 4.5–6, whereas that of soda lime glasses is near 7. The pipet capacitance generates noise by several mechanisms that will be described later. The dissipation factor is a measure of the lossiness of a dielectric material. Ideal capacitors display no dielectric loss and do not generate thermal noise. However, all real dielectrics are lossy and do produce thermal noise; we refer to this as dielectric noise. Glasses with the lowest dissipation factors are the least lossy and generate the least dielectric noise. Quartz is among the least lossy of all practical dielectrics; its dissipation factor, which is in the range of $10^{-5}$–$10^{-4}$, is far lower than that of other glasses used for patch pipets. Several high-lead glasses have dissipation factors of $\sim 10^{-3}$. The dissipation factor of borosilicates that have been used successfully to fabricate patch pipets varies from about 0.002–0.005. Soda lime glasses have the highest dissipation factor (~0.01), which is the principal reason for their high noise.

The best glasses for patch pipet fabrication are those with the best electrical properties, i.e., low dissipation factor and low dielectric constant. However, understanding pipet noise requires more than simply understanding the electrical properties of glass. A variety of other factors also influence the noise performance of the patch pipet, e.g., pipet geometry, depth of immersion, and the type and extent of elastomer coating. Here we will summarize our present understanding of all major pipet noise sources; more detailed discussions can be found elsewhere (Levis and Rae, 1992, 1993; Rae and Levis, 1992a,b).

Attaching the electrode holder to the headstage input will slightly increase noise above its minimum level associated with an open circuit input. The mechanisms involved in generating this noise are discussed elsewhere (Levis and Rae, 1993). Here we only note that the contribution of the holder by itself to total patch-clamp noise should be very small. Holder noise is minimized by constructing the holder from low-loss dielectric materials, minimizing its size, and always keeping it clean. Shielded holders will produce more noise than unshielded holders.

Simply adding the pipet to the holder (attached to the headstage input) slightly increases the capacitance at the amplifier input. After the pipet has been immersed into the bath and a GΩ seal has been formed, the capacitance at the headstage input is further increased. As will be seen, the capacitance of the immersed portion of the pipet is a consideration in several sources of noise. Here, however, we begin by noting that all of this capacitance will at minimum produce noise because it is in series with the input voltage noise, $e_n$, of the headstage amplifier. The current noise produced has a power spectral density (PSD, Amp$^2$/Hz) with rises as $f^2$ at frequencies above roughly 1 kHz. Of course, this noise is correlated with noise arising from $e_n$ in series with other capacitance (amplifier input capacitance, stray capacitance, capacitance of the electrode holder). The total amount of capacitance associated with an immersed pipet can vary from a fraction of a pF up to 5 pF or more. Low capacitance is associated with heavy elastomer coating and shallow depths of immersion. Obviously, the amount of noise arising from this mechanism increases as the capacitance associated with the pipet increases. However, regardless of the value of the pipet capacitance, the noise it contributes in conjunction with $e_n$ will be small in comparison with other pipet noise sources described later. For low-noise patch-clamp measurements, it is imperative that the pipet capacitance be minimized. The reason for this will become more clear as other noise sources associated with this capacitance are described.

In addition to the mechanism just described, and to noise arising from the membrane to glass seal (which will be discussed separately), the pipet contributes noise by at least four mechanisms. Each mechanism will be described later, followed by a summary of pipet noise sources. Our emphasis is on the minimization of each noise, rather than simply its description.

### 5.2. Thin-Film Noise

Thin films of solution are capable of creeping up the outer surface of the pipet from the bath (Fig. 1A). The noise associated with such films has previously been shown to

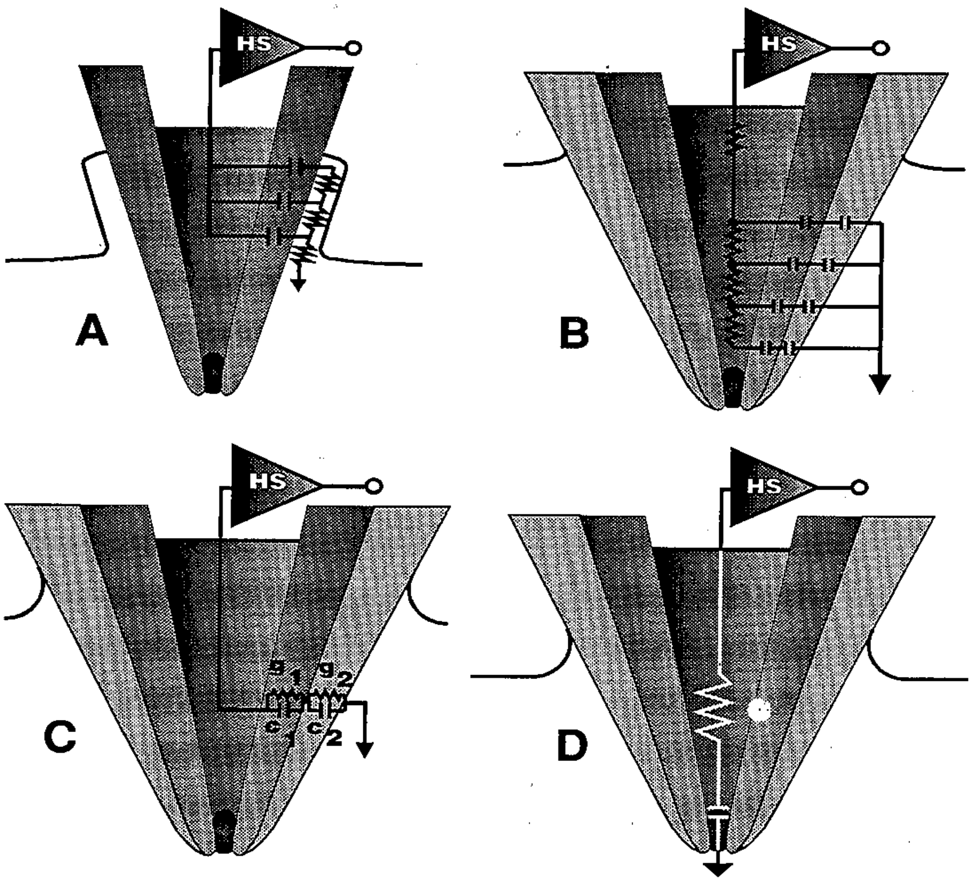

Fig. 1. Simplified circuit representations of the major noise mechanisms of the patch pipet. **(A)** Thin-solution film on the exterior surface of an uncoated patch pipet; noise arises from the thermal voltage noise of the distributed resistance of this film in series with the capacitance of the pipet wall. In **(B–D)**, the pipet is shown coated with a suitable elastomer. (B) Distributed RC noise arising from the thermal voltage noise of the distributed resistance of the pipet filling solution in series with the distributed capacitance of the immersed portion of the pipet wall and its elastomer coating. (C) Dielectric noise of the series combination of the pipet ($\gamma_1$, $C_1$, where $\gamma_1 = \omega C_1 D_1$) and the elastomer coating ($\gamma_2$, $C_2$, where $\gamma_2 = \omega C_2 D_2$). In the region immersed in the bath, the glass wall of the pipet and its elastomer coating are represented by ideal lumped capacitances $C_1$ and $C_2$, respectively in parallel with loss conductances $\gamma_1 = 2\pi f C_1 D_1$ and $\gamma_2 = 2\pi f C_2 D_2$. The thermal noise (dielectric noise) of the coated pipet is then $4kT$ multiplied by the real part of the admittance of the series combination of dielectrics. (D) $R_e$-$C_p$ noise arising from the thermal voltage noise of the entire (lumped) resistance, $R_e$ of the patch pipet in series with the patch capacitance, $C_p$. *See text* for further details.

be very significant (Hamill et al., 1981). Such a film will have a relatively high distributed resistance, and the thermal voltage noise of this resistance is in series with the distributed capacitance of the pipet wall. It is expected that the PSD of this noise will rise at low to moderate frequencies and then level out at frequencies in the range of several kHz to several tens of kHz. We have estimated with uncoated pipets made from several types of glass that the noise associated with such a film of solution is usually in the range of 100–300 pA rms in a bandwidth of 5 kHz. Evidence for such films has been found in pipets fabricated from all glasses we have tested when elastomer coating has been omitted. However, pipets pulled from GE quartz produce significantly less noise without elastomer coating than any other type of glass. Apparently the surface of this glass is less subject to the formation of such thin films.

Coating the pipet with Sylgard 184 or other suitable elastomers can essentially eliminate the formation of external films of solution and eliminate the otherwise large amounts of noise they produce. These elastomers have a hydrophobic surface that prevents the formation of such films. Sylgard 184 is so effective in this regard that we have been unable to detect any thin-film noise in properly coated pipets.

Thin films of solution may also be able to form on the interior surface of the pipet and inside the holder. To avoid the formation of such films, it is possible after filling the pipet with the desired amount of ionic solution to layer a few millimeters of paraffin oil or silicone fluid on top of the filling solution. However, we have found that this is usually unnecessary (and it can get messy) if excess solution is carefully suctioned from the back of the pipet as described earlier.

### *5.3. Distributed RC Noise*

Noise will also arise from the thermal voltage noise of the resistance of the pipet filling solution in series with the capacitance of the immersed portion of the pipet (Fig. 1B).

Most of the resistance of the pipet resides at or near its tip. However, significant resistance is distributed along the shank distal to the tip. This resistance (and its thermal voltage noise) are in series with the capacitance of the pipet wall distributed along the portion that is immersed in the bath. We refer to noise that results as distributed RC noise. In the frequency range of greatest interest to patch-clamping (DC to 100 kHz or more), the PSD of this noise is expected to rise as $f^2$. Our theoretical predictions of the noise arising from this mechanism (e.g., Levis and Rae, 1992) have relied on idealizations of the pipet geometry. More complicated real-world geometrics and factors such as nonuniform thinning of the pipet wall that often occurs during pulling are expected to make such predictions rather imprecise. Because of this, we chose to study distributed RC noise directly. These experiments used quartz pipets pulled from OD/ID = 2.0 tubing that were coated with Sylgard 184 only to the point where the electrode entered the bath (i.e., most or all of the immersed portion of the pipet was uncoated); immersion depth was ~1.8 mm, and the pipets were sealed to Sylgard (seal resistance >200 GΩ). Our strategy was to vary the ionic strength of the internal filling solution. Changing the ionic strength of the filling solution will change the pipet resistance, but it will have no effect on the pipet capacitance. Because of this, it is expected that for pipets of equivalent geometry and with the same depth of immersion into the bath, the PSD of distributed RC noise will vary as 1/M, where M is the ionic concentration of the filling solution. The rms noise in any particular bandwidth is expected to vary as $1/M^{1/2}$. In our study of this noise, we used NaCl solutions with concentrations from 1.5 m*M* to 1.5*M* to fill the pipet. As expected, the noise increased as the ionic strength of the filling solution decreased. When the noise component attributable to distributed RC noise was parsed from total noise (and it was the dominant noise source for ionic strength of 15 m*M* or less), the predicted behavior was reasonably well confirmed. Also, as expected, the PSD of this noise component increased approximately as $f^2$ as frequency increases.

On the basis of these experiments, we concluded that for uncoated quartz pipets that were pulled from OD/ID = 2 tubing and immersed to a depth of about 1.8 mm and filled with 150 m*M* NaCl (i.e., the ionic strength typical of most experiments), the PSD of distributed RC noise was approximated by $2.5 \times 10^{-38} f^2$ amp$^2$/Hz. The rms noise contribution in a bandwidth $B$ is then $(8 \times 10^{-39} c_3 B^3)^{1/2}$ amps rms, where $c_3$ is a coefficient that depends on the type of filter used ($c_3 \approx 1.9$ for an 8-pole Bessel filter). This equation predicts a noise component of ~44 pA rms for a 5 kHz bandwidth (–3 dB, 8-pole Bessel filter), or about 123 fA rms in a 10-kHz bandwidth. It must be remembered, however, that these results were for relatively thick-walled pipets fabricated from quartz, which has a low dielectric constant of 3.8. It must also be remembered that the pipets were not coated with Sylgard (or other suitable elastomer) in the region immersed in the bath. The capacitance of the wall of the pipet is expected to vary directly with the dielectric constant of the glass (for pipets of the same geometry) and vary inversely roughly in proportion to the log of the OD/ID ratio. The PSD of distributed RC noise should vary in proportion to the pipet capacitance ($C_e$) squared; rms noise in a given bandwidth will therefore vary linearly with $C_e$. Thus, for an uncoated pipet fabricated from OD/ID = 1.4 tubing from a glass with a dielectric constant of 7.6 (twice that of quartz), the numbers given above would be expected to increase by a factor of about 4. On the other hand, coating the immersed portion of a pipet with a suitable elastomer will thicken its walls and therefore reduce $C_e$. Thus, very heavy coating of the pipet with an elastomer, such as Sylgard 184, can dramatically reduce distributed RC noise, and, with such coating, the amount of this noise will become almost independent of the type of glass used. In the experiments described earlier, we measured $C_e$ to be in the range of 1.4–1.8 pF. We have found that using the tip-dip elastomer coating method (Levis and Rae, 1993) to build up a heavy coat of Sylgard all the way to the tip of the pipet, we can obtain values of $C_e$ as low as ~0.35 pF for a comparable depth of immersion. This should reduce distributed RC noise to <10 fA rms in a 5-kHz bandwidth. Of course, shallow depths of immersion can also reduce distributed RC noise.

From the preceding discussion, it should be clear that the reduction of distributed RC noise is one of the major benefits of coating the immersed portion of the pipet with a low dielectric constant elastomer such as Sylgard 184. This noise component can also be minimized by using thick-walled tubing of glasses with low dielectric constants and by shallow depths of immersion of the pipet into the bath. Distributed RC noise is also expected to depend on pipet geometry, and should be minimized by shapes that reduce the distributed resistance distal to the pipet tip.

### 5.4. Dielectric Noise

Dielectric noise (Fig. 1C) will also arise from the capacitance of the pipet wall over the region that is immersed in the bathing solution. For pipets fabricated from glasses other than quartz, dielectric noise is likely to be the dominant source of noise arising from the pipet. For a single dielectric with a capacitance $C_d$ and a dissipation factor $D$, the PSD of dielectric noise is given by:

$$S_d^2 = 4kTDC_d(2\pi f) \text{ Amp}^2/\text{Hz} \qquad (1)$$

The rms noise in a bandwidth $B$ is given by:

$$I_d = (4kTDC_d c_2 \pi B^2)^{1/2} \text{ Amp rms} \qquad (2)$$

where k is Boltzman's constant and $T$ is absolute temperature (°K). $c_2$ Is a coefficient that depends on the type of filter used; for an 8-pole Bessel filter with $B$ as the –3-dB bandwidth, $c_2 \approx 1.3$. It is important to note that the PSD of dielectric noise rises linearly with increasing frequency and that the rms value of this noise is proportional to filter bandwidth. This is quite unlike the other noise sources discussed, and is very useful in experimentally parsing dielectric noise from other types of noise generated by the pipet.

For an uncoated pipet, these equations can be applied simply by noting that $C_d$ is the capacitance of the immersed portion of the pipet (denoted by $C_e$ above), and that $D$ is the dissipation factor of the glass. It is instructive to consider two uncoated pipets with the same geometry both pulled from OD/ID = 1.4 tubing and both immersed to a depth of about 2 mm. One pipet is fabricated from quartz ($D = 0.0001$, dielectric constant = 3.8) and the other pipet is fabricated from a borosilicate with $D = 0.005$ and a dielectric constant of 5.0. The capacitance ($C_d$ or $C_e$) of the quartz pipet should be about 1.5 pF, whereas that of the borosilicate pipet will be about 2 pF because of its higher dielectric constant. Using these numbers, it can be estimated that the uncoated quartz pipet will produce about 16 fA rms dielectric noise in a 5-kHz bandwidth (–3 dB, 8-pole Bessel filter), whereas the borosilicate pipet would produce 128 fA rms dielectric noise in the same bandwidth. The superiority of quartz is clear in this case.

Of course, the importance of coating the pipet with a suitable elastomer has already been demonstrated, regardless of the type of glass used. Therefore, it is necessary to consider the dielectric noise in this more complicated situation. We have presented a more detailed analysis of the dielectric noise in this case elsewhere (Levis and Rae, 1993). Here, we will summarize our most important conclusions. When the pipet is coated with an elastomer, it is necessary to derive equations that describe the dielectric noise of the series combination of two different dielectrics with capacitances $C_1$ and $C_2$ and dissipation factors $D_1$ and $D_2$ (*see* Fig. 1C and its legend). For $D_1, D_2 << 1$, the dielectric noise PSD of the elastomer coated pipet is well approximated by:

$$4kT(2\pi f)[(D_1C_1C_2^2 + D_2C_2C_1^2)/(C_1 + C_2)^2]\ \mathrm{Amp}^2/\mathrm{Hz} \qquad (3)$$

and the rms noise in a bandwidth B is approximated by:

$$\{4kTc_2\pi\beta^2[(D_1C_1C_2^2 + D_2C_2C_1^2)/(C_1 + C_2)^2]\}^{1/2}\ \mathrm{Amps\ rm} \qquad (4)$$

In these equations $C_1$ and $D_1$ are the capacitance and dissipation factor of the glass wall of the pipet and $C_2$ and $D_2$

are the capacitance and dissipation factor of the elastomer coating. The capacitance $C_1$ depends on the depth of immersion, the thickness of the pipet wall, and the dielectric constant of the glass. For a 2-mm depth of immersion, $C_1$ can vary from as little as about 1 pF for very thick-walled quartz pipets, to more than 6 pF for thin-walled pipets made from glassses with high dielectric constants (e.g., soda lime and high-lead glasses). Of course, the capacitance $C_2$ of the elastomer coating also depends on the depth of immersion, the dielectric constant of the elastomer, and the thickness of the elastomer coating. Obviously, heavy elastomer coating will lead to the smallest values of $C_2$. However, it is important to realize that the thickness of the elastomer coating will not be uniform. In particular, it is hard to achieve very thick elastomer coatings near the tip of the pipet. The dip method of elastomer coating (Levis and Rae, 1993) has proved to be useful in building up relatively heavy coats of elastomer all the way to the tip of the electrode, but even with this method the thickness of the coat is still not uniform. Because of this, it is difficult to predict the value of $C_2$. However, we have measured the value of $C_2$ (*see* Levis and Rae, 1993) to be as little as 0.4–0.5 pF for a 2-mm immersion depth when heavy coatings of Sylgard were applied with the dip method. With lighter coating, the value of $C_2$ can easily be much higher (2 pF or more).

The dissipation factor $D_1$ of the glasses used to fabricate patch pipets have already been discussed; reported values range from as little as $10^{-5}$–$10^{-4}$ for quartz to as much as 0.01 for soda lime glasses. The dissipation factor $D_1$ of the elastomer is also very important. Sylgard 184 has a dissipation factor of about 0.002, which is lower than that of most glasses, with the notable exception of quartz. Because of this, coating pipets fabricated from glasses other than quartz will significantly reduce their dielectric noise and the relative reduction will be greatest for the poorest (most lossy) glasses. However, the dissipation factor of Sylgard 184 is a factor of 20 or more higher than that of quartz, and predictions based on Eqs. (3) and (4) indicate that for all realistic values of $C_2$

coating a quartz pipet with Sylgard will actually increase its dielectric noise relative to that which would have been produced by the pipet alone. This is true despite the reduction in overall capacitance produced by the Sylgard coating. Thus, for a quartz pipet with $C_1 = 1.5$ pF and $D_1 = 0.0001$ and a Sylgard coating with $C_2 = 0.5$ pF and $D_2 = 0.002$, Eq. (4) predicts 30 fA rms of dielectric noise in a 5-kHz bandwidth (–3 dB, 8-pole Bessel filter), whereas as described earlier, the same pipet without the Sylgard coating would have produced only about 17 fA rms dielectric noise in this bandwidth. Estimates of the dielectric noise of quartz pipets coated with Sylgard and several similar elastomers and sealed to Sylgard have produced values that are in good agreement with the predictions of Eqs. (3) and (4).

It is apparent from the earlier discussion that coating a quartz patch Pipet with Sylgard 184 is not desirable in terms of dielectric noise. Nevertheless, coating with Sylgard or some other suitable elastomer is necessary to eliminate thin-film noise and to minimize distributed RC noise. If fact, very heavily Sylgard-coated quartz pipets display the least noise of all pipets, so the small increment in dielectric noise resulting from such coating is more than offset by the benefits in terms of reduction of other types of noise. It is also important to realize that even though Sylgard-coating a quartz pipet will increase its dielectric noise, the final dielectric noise of such a pipet still remains significantly below that of Sylgard-coated pipets fabricated from any other type of glass we have tested. If elastomers with dissipation factors significantly less than that of Sylgard 184 can be found that are otherwise suitable for coating pipets, they could be effective in lowering the dielectric noise of quartz pipets. It can be appreciated from examination of Eqs. (3) and (4) that the dissipation factor of such an elastomer need not be less than that of quartz to lower total dielectric noise of a heavily coated pipet. Such elastomers (if found) should also be very beneficial for other types of glass. Dow Corning R-6101 and Q1-4939 both are reported by the manufacturer to have dissipation factors of 0.00025. However, our preliminary measurements of pipets coated with R-6101 have failed to demonstrate any significant advantage over pipets coated with similar thicknesses of Sylgard 184. Although we are unable to account for this finding, it certainly seems possible that the true dissipation factor of this elastomer exceeded the value in the manufacturer's data sheet.

Because of the volume of material presented regarding dielectric noise, it is probably worthwhile to summarize our conclusions. For thick-walled quartz pipets with heavy Sylgard coating to the tip and seal to Sylgard at an immersion depth of ~2 mm, our estimates of dielectric noise has generally been in the range of 20–35 fA rms in a 5-kHz bandwidth. On occasion, with actual excised patches and heavily Sylgard-coated patch pipets, our estimates of dielectric noise have been <15 fA rms in a 5-kHz bandwidth when the electrode tip has been withdrawn close to the surface of the bath. For other types of glasses, dielectric noise is significantly higher. Our previous measurements of the noise arising from light to moderately Sylgard-coated pipets made from more than 20 different glasses (Rae and Levis, 1984, 1992a), indicated that in a 5-kHz bandwidth and with a ~2-mm depth of immersion dielectric noise varied from about 100–200 fA rms. The lowest noise was associated with glasses with the smallest loss factor (i.e., dissipation factor multiplied by dielectric constant), whereas the highest noise arose from the very lossy soda lime glasses. Recently, we have measured a few pipets made from Corning 7052 and 7760 (tubing OD/ID = 1.4) that were heavily coated with Sylgard 184 to the tip by the dip method described earlier. These measurements indicated that dielectric noise could be as low as ~70 fA rms in a 5-kHz bandwidth for these glasses (with heavy Sylgard coating) at a 2-mm immersion depth. This is somewhat more noise than would be predicted from Eqs. (3) and (4), but less than we have estimated previously.

### 5.5. $R_e$-$C_p$ *Noise*

The last pipet noise mechanism that we will consider is the noise that is expected to arise from the thermal voltage noise of the entire lumped pipet resistance, $R_e$, in series with the capacitance of the patch membrane, $C_p$; we refer to this noise source as $R_e$-$C_p$ noise (*see* Fig. 1D). This noise is expected to have a PSD that increases as $f^2$ up to frequencies of about $1/2\pi R_e C_p$ (which is usually several hundred kHz); at frequencies below this the PSD is expected to be:

$$S_{ep}{}^2 = 4\pi^2 e_e{}^2 C_p{}^2 f^2 \text{ Amp}^2/\text{Hz} \tag{5}$$

where $e_e{}^2 = 4kTR_e$, i.e., the thermal voltage noise PSD for the pipet. The rms noise attributable to this mechanism in a bandwidth $B$ is then given by:

$$I_{ep} = (1.33\pi^2 c_3 e_e{}^2 C_p{}^2 B^3)^{1/2} \text{ Amps rms} \tag{6}$$

where $c_3$ is a coefficient that again depends on the type of filter used to establish the bandwidth; for an 8-pole Bessel filter with a –3-dB bandwidth of $B$ Hz, $c_3 \approx 1.9$.

For single-channel measurements, patch capacitance typically ranges from approx 0.01–0.25 pF for pipet resistances in the range 1–10 MΩ (Sakmann and Neher, 1983). As expected, higher values of patch capacitance are associated with lower resistance pipets. Because of the inverse relationship between $R_e$ and $C_p$, Eqs. (5) and (6) predict that the smallest amount of $R_e$–$C_p$ noise will arise from the smallest patches, even though such patches are obtained with higher resistance pipets. For example, with $R_e$ = 10 MΩ and $C_p$ = 0.01 pF, Eq. (6) predicts a noise contribution of only about 6 fA rms in a 5-kHz bandwidth (–3 dB, 8-pole Bessel filter). On the other hand, with $R_e$ = 2 MΩ and $C_p$ = 0.25 pF, the predicted noise is more than 60 fA rms in the same bandwidth. This latter amount of noise can exceed the total of all other pipet noise sources for quartz pipets, and remains significant even for pipets fabricated from other glasses. Obviously, however, this noise source itself does not depend on the type of glass, but rather on the geometry of the pipet (and, to some extent, on luck).

### 5.6. Seal Noise

The noise associated with the membrane-glass seal is less easily predicted. It is expected that the PSD of this noise for zero applied voltage will be given by $4kT\ Re\{Y_{sh}\}$, where $Re\{Y_{sh}\}$ is the real part of the seal admittance. The minimum value of $Re\{Y_{sh}\}$ is $1/R_{sh}$, where $R_{sh}$ is the DC seal resistance, and this leads to a minimum estimate of the seal noise in a bandwidth $B$ of $(4kTB/R_{sh})^{1/2}$. This is just the thermal current noise of the seal resistance and can be very small for high resistance seals. For example, for a 200 GΩ seal and a bandwidth of 5 kHz, $(4kTB/R_{sh})^{1/2} = 20$ fA rms. Our measurements from several patches with seal resistances in the range 40–100 GΩ have shown that the noise attributable to the seal is often indistinguishable from the predicted thermal current noise of $R_{sh}$ (Rae and Levis, 1992b). Nevertheless, it is certainly possible that seal noise may sometimes exceed this minimum prediction. As anyone who has spent much time trying to achieve low noise with the patch-clamp technique knows, there is a great deal of variability in the noise achieved even when all of the precautions we have described have been followed and when very high seal resistances have been obtained. It is certainly tempting to blame some of this variability on the noise associated with the seal.

## 5.7. Summary of Pipet Noise Sources

It is important to realize that the noise sources described above (with the exception of noise arising from various capacitances in series with the amplifier's input voltage noise $e_n$) are all uncorrelated. Uncorrelated noise sources add in an rms fashion. For example, if four uncorrelated noise sources have rms values denoted by $E_1$, $E_2$, $E_3$, and $E_4$, then the total rms noise resulting from the summation of these sources is given by $(E_1^2 + E_2^2 + E_3^2 + E_4^2)^{1/2}$. Because of this, the largest individual source of noise will tend to dominate total noise.

Of the noise sources described above, only thin-film noise can be completely eliminated (or in any case reduced to negligible levels). Distributed RC noise, dielectric noise, and $R_e$-$C_p$ noise can never be eliminated, but they can be minimized. In many cases, precautions taken to reduce one noise source will also be beneficial in reducing other sources of noise. Thus, with any type of glass, thick-walled pipets will, all else being equal, have less capacitance, and therefore display less distributed RC noise and dielectric noise. Similarly, shallow depths of immersion will also reduce pipet capacitance and simultaneously reduce distributed RC and dielectric noise. Coating pipets with a heavy layer of a low-loss elastomer such as Sylgard 184 will also reduce the pipet's capacitance and reduce distributed RC noise for all types of glass. For all types of glass other than quartz, a heavy coat of Sylgard 184 extending as close to the tip as possible will also significantly reduce dielectric noise. In the case of quartz pipets, elastomers with dissipation factors comparable to that of Sylgard 184 will actually somewhat increase dielectric noise. However, within the range of realistic thicknesses of the coating, even for quartz, heavy coatings will generally lead to the least dielectric noise; this is compatible with the requirements for minimizing distributed RC noise in quartz pipets. It is also important to recall that even though Sylgard coating somewhat increases the dielectric noise of quartz pipets, the final dielectric noise of a heavily Sylgard-coated quartz pipet is still much less than that of pipets made from any other type of glass. The major distinction in terms of noise between pipets fabricated from quartz and other types of glasses is, in fact, the much lower dielectric noise of quartz. $R_e$-$C_p$ noise often has been ignored in the past, and in many situations it is sufficiently small to still be ignored. However, when all other sources of noise successfully have been reduced to the lowest limits presently achievable, it can become significant, and even dominant at very wide bandwidths (Levis and Rae, 1993). $R_e$-$C_p$ noise is minimized by forming the smallest patch areas that are consistent with the goals of the experiment being undertaken. Although the data are widely scattered, patch area (or patch capacitance) decreases as pipet resistance increases. The net result is that it is predicted that higher resistance patch pipets with small tips will tend to produce the least amount of $R_e$-$C_p$ noise. The geometry of such electrodes is not necessarily the best selection for minimizing distributed RC noise, but this can be overcome by heavy elastomer coating. Although we have not systematically studied the relationship between pipet resistance (tip diameter) and noise, it is our experience that the lowest noise patches are usually obtained from small-tipped high resistance pipets.

It is difficult to assign values to what can be expected as "typical" or "best-case" noise from pipets fabricated from different glasses in different situations. Nevertheless, some rough estimates can be provided. For low-loss borosilicate, aluminosilicate, or high-lead glasses with moderate Sylgard coating extending to within ~100 µm of the tip, it is reasonable to expect that in a 5-kHz bandwidth total pipet noise (excluding seal noise) as low as 100–120 fA rms can be achieved with a ~2-mm depth of immersion. With very heavy Sylgard coating all the way to the tip, this value should fall to somewhat less than 100 fA rms in this bandwidth. With quartz pipets that are heavily Sylgard-coated to the tip we have been routinely able to keep total pipet noise to ~40-fA rms in a 5-kHz bandwidth for a 2-mm immersion depth. With the quartz pipet raised to the surface of the bath with an excised membrane patch, occasionally we have achieved a total noise of the pipet plus seal as low as 30–35 fA rms in a 5-kHz bandwidth; subtracting the thermal current noise of the seal (as judged from its measured resistance) yields an estimate of 10–20 fA rms for total pipet noise in these cases.

## 5.8. Noise Sources for Whole-Cell Voltage Clamping

Whereas all of the pipet noise mechanisms described earlier, with the exception of $R_e$-$C_p$ noise, are present in whole-cell voltage clamping, their relative importance is very much less than is the case for patch voltage clamp measurements. Of course, this is not because these pipet noise sources have become less in the whole-cell situation, but rather because other noise sources have become much higher. In the first place, most whole-cell voltage clamp measurements are made with a patch-clamp headstage amplifier configured with a 500-MΩ feedback resistor. In a 5-kHz bandwidth, this resistor alone will produce 400-fA rms noise, which is more than even soda lime pipets will produce provided they are reasonably Sylgard-coated. Under most situations, however, the dominant source of noise in a whole-cell voltage clamp will be the thermal current noise of the pipet resistance $R_e$ in series with the cell membrane capacitance $C_m$.

As just noted, the whole-cell voltage clamp lacks $R_e$-$C_p$ noise. The reason for this is simply that the patch membrane has been disrupted, or shorted out, as is the case for perforated patch measurements. However, in the whole-cell situation, the entire cell membrane is in series with the pipet resistance and with the thermal voltage noise of this resistance. The noise produced by this has precisely the same mechanism that underlies $R_e$-$C_p$ noise, but, since $C_m >> C_p$, it is of far greater magnitude. It might also be recalled that the time constant $R_eC_p$ will typically be 1 µs or less and so usually can be neglected. However, the time constant $R_eC_m$ is much larger and its effects can not be ignored, either in terms of noise or dynamic performance.

Of course, the electrode resistance $R_e$ is the series resistance in the whole-cell variant of the patch voltage clamp, and many of its effects are well known and need no further comment here. But it seems that some of its effects can never be emphasized often enough. One of these is the filtering effect that uncompensated series resistance has on the measured current. In the absence of series resistance compensation, this filtering effect (equivalent to a simple RC low-pass filter) limits the actual bandwidth of current measurement to $1/2\pi R_eC_m$. For example, with $R_e = 10$ MΩ and $C_m = 50$ pF, this is ~320 Hz, and it should be remembered that $R_e$, after patch disruption or perforation, usually is higher than the pipet resistance that was measured in the bath. With series resistance compensation, this bandwidth limit is increased. We will define as the fraction of the series resistance compensated ($0 < \alpha < 1$), and $\beta = 1 - \alpha$. With series resistance compensation, the uppermost usable bandwidth is extended to $1/2\pi\beta R_eC_m$. So in the previous example, 90% series resistance compensation ($\beta = 0.1$) will extend the actual bandwidth limit to about 3.2 kHz. It will also greatly increase the noise at this bandwidth. The PSD, $S_{em}{}^2$, of noise arising from the thermal voltage noise of $R_e$ in series with $C_m$ is given by:

$$S_{em}{}^2 = (4\pi^2 e_e{}^2 C_m{}^2 f^2)/(1 + 4\pi^2\beta^2 R_e{}^2 C_m{}^2 f^2) \qquad (7)$$

where $e_e{}^2 = 4kTR_e$ is the thermal voltage noise PSD of $R_e$. Note that this expression takes into account the effects of series resistance compensation. For 100% series resistance compensation ($\alpha = 1$, $\beta = 0$), Eq. (7) reduces to $4\pi^2 e_e{}^2 C_m{}^2 f^2$, which has exactly the same form as Eq. (5).

From Eq. (7) it can be seen that the PSD of the noise arising from $R_e$ and $C_m$ rises with increasing frequency as $f^2$ until it reaches $f = 1/2\pi\beta R_e C_m$.

Thereafter, this noise plateaus to a value of $4kT/\beta^2 R_e$, which, of course, is many times larger than the thermal current noise of the feedback resistor. This plateau level of the PSD will be maintained until a frequency is reached where it is rolled off by an external filter (or the inherent bandwidth limit of the electronics). As an example of the magnitude of the noise introduced by this mechanism, consider a favorable example for whole-cell voltage clamping with $R_e = 5$ MΩ and $C_m = 30$ pF. Without series resistance compensation, the "corner frequency" at which the noise PSD plateaus (and the limit of actual bandwidth of current measurement) is about 1060 Hz. For a –3-dB bandwidth (8-pole Bessel filter) of current measurement only 500 Hz, the noise arising from $R_e$ and $C_m$ would already be nearly 0.5 pA rms, which is more than a very bad electrode would produce in a bandwidth of 5 kHz. By a bandwidth of 1 kHz, the noise would have increased to about 1.3 pA rms. Increasing the bandwidth of current measurement much beyond 1 kHz without series resistance compensation is not justified, since the measured current will still be effectively filtered at 1.06 kHz (–3-dB bandwidth of the 1-pole low-pass filter arising from $R_e$ and $C_m$). This does not mean, however, that setting the external filter to a bandwidth higher than 1 kHz will not add more noise. Increasing the bandwidth of the external filter to 5 kHz will increase the noise to more than 3 pA rms, but it will provide very little signal information that was not contained when the data was filtered at 1 kHz. Series (pipet) resistance compensation can extend the usable bandwidth, but, of course, it will significantly increase the noise at eternal filter bandwidths higher than $1/2\pi R_e C_m$. Thus, with 90% series resistance compensation, the maximum usable bandwidth of current measurement is extended to 10.6 kHz. In this case, with an external filter (8-pole Bessel) with a –3-dB bandwidth of 5 kHz, the noise is increased to almost 15 pA rms. For a 10-kHz bandwidth the noise will increase to about 40 pA rms. In noises of this magnitude, the pipet noise mechanisms previously discussed become quite insignificant. It can therefore be concluded that many of the characteristics of the pipet that were important to patch-clamping are not important to a whole-cell voltage clamp situation.

The noise arising from $R_e$ and $C_m$ in whole-cell voltage clamping can only be minimized by minimizing $R_e$ and/or $C_m$. Of course, minimizing $C_m$ means selecting small cells and often this is not possible. In addition, it should also be noted that if you are studying a particular type of channel in a population of cells of various sizes but the channel density is the same in all cases, there is no clear advantage in terms of signal-to-noise ratio of selecting smaller cells. For a constant value of $R_e$ it is simple to show that at a given bandwidth (below $1/2\pi\beta R_e C_m$) the rms noise will decrease linearly as $C_m$ decreases, but, since the number of channels is also proportional to $C_m$, the signal will also decrease linearly with decreasing $C_m$: Signal-to-noise ratio will be constant. In this case, signal-to-noise ratio only depends on $R_e$ and it will improve as $1/R_e^{1/2}$. So the most practical way to minimize this source of noise is to use the lowest resistance pipets that are capable of sealing to your cells and make every effort to minimize the increase in access resistance that often occurs when the patch is disrupted.

Finally, it is worth emphasizing that another important way of minimizing this noise is to not make the mistake of using a bandwidth of the external filter that is not justified by the situation. Increasing the external bandwidth significantly beyond $1/2\pi\beta R_e C_m$, essentially adds no information about the signal, but it will add additional noise.

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