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The Use of Quartz Patch Pipettes for Low Noise Single Channel Recording

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ABSTRACT Quartz has a dissipation factor of $\sim 10^{-4}$, which is an order of magnitude less than that of the best glasses previously used to fabricate patch pipettes; it's dielectric constant of 3.8 is also lower than that of other glasses. On the basis of these electrical characteristics it is expected that patch pipettes pulled from quartz tubing will produce significantly less noise than pipettes made from other glasses. Our work confirms these expectations and we describe theoretical and practical aspects of the use of quartz pipettes for single channel patch voltage clamp measurements. Methods for pulling quartz pipettes with a laser-based puller and coating them with low-loss elastomers are discussed, as are precautions that are necessary to achieve low noise recordings. We have shown that quartz pipettes can be pulled from tubing with outer diameter to inner diameter ratios as large as 3 and a method of applying heavy elastomer coatings all the way to the tip of pipettes is presented. Noise sources arising from the pipette and its holder are described theoretically, and it is shown that measured noise is in good agreement with such predictions. With low noise capacitive feedback electronics, small geometry holders, and thick-walled quartz pipettes coated with low-loss elastomers we have been routinely able to achieve noise of 100 fA rms or less in a 5-kHz bandwidth with real cell patches and a pipette immersion depth of ~2 mm. On occasion we have achieved noise as low as 60 fA rms in this bandwidth.

INTRODUCTION

The background noise in a single channel patch clamp measurement determines the maximum bandwidth that will preserve an adequate signal-to-noise ratio. At a fixed bandwidth, background noise determines the minimum conductance channel which can be studied. Obviously, high resolution measurements require that interference from external noise sources such as line frequency pick-up be reduced to a minimum. Aside from such external interference, noise in patch clamp measurements arises from a variety of sources. These include the headstage amplifier, the pipette and its holder, and, of course, the membrane-to-glass seal. In the past, the patch electrode has been a major, if not dominant, source of noise. Here we show that by the use of thick-walled quartz pipettes coated with low-loss elastomers, the noise of the pipette can be reduced to the point where it is smaller than that of even the very best present-day electronics. By using capacitive feedback electronics, small geometry holders fabricated from low-loss dielectrics, and coated quartz electrodes, we have been able to routinely make single channel measurements with background noise levels less than 100 fA rms in a 5-kHz bandwidth. On occasion, noise levels as low as 60 fA rms have been achieved in this bandwidth, which is only about 30% greater than the noise of the electronics themselves.

It has been obvious for some time that quartz possesses excellent electrical properties that should allow it to produce patch pipettes with much lower noise than pipettes fabricated from any other type of glass. The capacitance of the pipette wall is involved in the generation of noise by several mechanisms that will be described in detail below. One major noise mechanism is related to the lossiness of the pipette's capacitance. Ideal capacitors are lossless and do not produce thermal noise. Unfortunately, however, all real dielectrics exhibit some loss which results in the generation of thermal noise (here called *dielectric noise*). The dissipation factor quantifies the dielectric loss of a material. The smaller the dissipation factor, the less lossy is the dielectric, and, for a given capacitance, the less the dielectric noise. The dissipation factor of quartz is more than an order of magnitude lower than that of the best glasses previously used for patch pipettes (Rae and Levis, 1984a, 1984b, 1992a). In addition, its dielectric constant is slightly lower than that of other glasses. However, until very recently it has not been possible to pull quartz tubing into patch pipettes because of its extremely high melting temperature. The introduction of a laser-based puller (P-2000; Sutter Instruments, Novato, CA) has allowed pipettes to be pulled from quartz tubing with outer diameter (OD) to inner diameter (ID) ratios as high as 3. The geometry of these pipettes can be controlled to be ideal for a variety of patch clamp applications.

In order to take full advantage of the low noise associated with quartz pipettes, it is necessary to minimize the noise contribution of the pipette holder and to use the very low noise electronics. In this paper, we will describe both theoretical and practical aspects of quartz patch pipettes. We describe the theoretical sources of noise associated with the holder and the pipette and present data which demonstrates both the adequacy of our predictions and the present limits of achievable noise performance with quartz pipettes. We

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also describe methods of pulling quartz pipettes and procedures for coating pipettes with several low-loss elastomers. A preliminary report of this work has been presented (Rae and Levis, 1993).

RESULTS

Patch clamp noise

In general, the total background noise power spectral density (PSD) for patch voltage clamp measurements can be described by:

$$S_t^2 = a_1 + a_2 f + a_3 f^2 \quad \mathrm{amp^2/Hz} \tag{1}$$

where f is the frequency in hertz. The noise variance in a bandwidth extending from DC to B (Hz) is then given by:

$$i_t^2 = c_1 a_1 B + c_2(a_2/2)B^2 + c_3(a_3/3)B^3 \quad \mathrm{amp^2} \tag{2}$$

and the rms noise in a bandwidth B is simply the square root of the variance, i.e.,

$$i_t = (c_1 a_1 B + c_2(a_2/2)B^2 + c_3(a_3/3)B^3)^{1/2} \quad \text{amps rms} \tag{3}$$

In Eqs. 2 and 3, the coefficients c_1, c_2, and c_3 depend on the type of filter used to establish the bandwidth. For a "brick wall" filter with a transfer function, $H(f)$ characterized by $H(f) = 1$ for $f \leq B$ and $H(f) = 0$ for $f > B$, all three coefficients are 1. However, for real filters with −3-dB bandwidths of B, these coefficients will exceed 1. Approximate values of these coefficients for two common filter types are as follows. Four-pole Butterworth: $c_1 \simeq 1.02$, $c_2 \simeq 1.1$, $c_3 \simeq 1.2$; eight-pole Bessel: $c_1 \simeq 1.04$, $c_2 \simeq 1.3$, $c_3 \simeq 1.9$.

Bessel filters are usually the best selection of readily available analog filter types for time domain measurements (Colquhoun and Sigworth, 1983; Levis and Rae, 1992). In this paper, we often present noise values using a four-pole Butterworth filter with a −3-dB bandwidth of 5 kHz, since this value is available on the front panel meter of an Axopatch 200A (Axon Instruments, Foster City, CA), the patch clamp amplifier used for these studies. These values would have to be increased by approximately 10–15% to correspond to results which would be obtained with an eight-pole Bessel filter.

Headstage noise

The lowest noise patch clamp "headstage" amplifiers utilize capacitive feedback. Such an amplifier replaces the traditional high valued (typically 50 GΩ) feedback resistor with a small capacitor (typically 1–2 pF), thereby forming an operational integrator which is followed by a differentiator to form a current-to-voltage converter. The only disadvantage of the capacitive feedback headstage is the need to periodically reset (i.e., discharge) the feedback capacitor. Further details of capacitive feedback amplifiers are provided elsewhere (Levis and Rae, 1992).

The power spectral density of the noise of the open-circuit headstage amplifier (Axopatch 200A) used in these experiments is well described by:

$$S_{\mathrm{hs}}^2 \simeq 9 \times 10^{-32} + 7 \times 10^{-35} f + 1.65 \times 10^{-38} f^2 \quad \mathrm{amp^2/Hz} \tag{4}$$

where f is the frequency in hertz. The white noise term (i.e., 9×10^{-32} amp²/Hz) arises primarily from the shot noise of the input JFET gate leakage current with a smaller component arising from leakage current associated with the reset FET. Total leakage current is equal to about 0.3 pA and produces noise that is equivalent to the thermal current noise of a 180-GΩ resistor. The f noise term arises primarily from dielectric noise associated with capacitors, packaging and the input capacitance of the JFET itself. The $1/f$ noise component of the input voltage noise, e_n, of the amplifier in conjunction with the capacitance associated with the headstage input also gives rise to current noise which rises linearly with frequency. The f^2 noise term is dominated by the white component of e_n which in conjunction with the capacitance at the headstage input produces current noise which rises in proportion to frequency squared. This capacitance consists primarily of the effective input capacitance of the JFET input stage, the feedback and compensation capacitors, plus stray capacitance; it totals approximately 11–12 pF for this amplifier. Voltage noise associated with the signals applied to the compensation capacitor and with the reset FET drive circuitry contribute a smaller component to this f^2 noise, as does the differentiator of the subsequent electronics. Headstage noise has been described in greater detail elsewhere (Rae and Levis, 1984a; Levis and Rae, 1992; Sigworth, 1983).

Electrode holder modifications

We use a modified HL-1-12 holder from Axon Instruments. The electrode hole is rebored to accept the 1.5-mm OD quartz tubing. The hole is extended to within 1 mm of the suction inlet. The bottom threads are extended also to within about 1 mm of the suction inlet. Then 2 mm are cut from the top threads and 3 mm from the bottom threads thus reducing the barrel length from 20 to 15 mm. The caps at both ends were also shortened. This shortening allows the internal Ag-AgCl wire to be reduced in length from 62 mm to about 41 mm. The suction line utilizes silastic tubing which we have found does not introduce extra noise.

Holder noise

The patch pipette holder represents a small but not insignificant source of noise in patch clamp measurements. In the first place, attaching the holder to the input of the headstage amplifier adds capacitance to this input which will react with the amplifier's input voltage noise, e_n, to produce a component of current noise whose power spectral density rises as f^2 (at frequencies beyond the $1/f$ range of e_n, i.e., beyond about 1 kHz). The capacitance added by the holder ranges from as little as ~0.6 pF for the small geometry designs described here to as much as about 1.5 pF for commercially available unshielded holders (unmodified HL-1-12 or HL-1-17 holders from Axon Instruments add about 1 pF of capacitance). Holders with metal shields can add more capacitance. The noise arising from this capacitance in series with e_n is perfectly correlated with noise arising from other capacitance associated with the headstage input (i.e., the JFET input capacitance, feedback and compensation capacitors, plus stray capacitance). The rms value (for a given bandwidth) of the total noise arising from e_n therefore increases in proportion to the increase in total capacitance; this is more than the sum-of-squares increase for uncorrelated noise sources. For a headstage amplifier with noise characteristics similar to that used in these investigations with an open circuit noise of 47 fA rms in a bandwidths of 5 kHz (−3 dB, four-pole Butterworth filter), the addition of 1.5 pF of capacitance to the headstage input would increase the noise to approximately 50 fA rms in the same bandwidth. 0.6 pF of added capacitance (equivalent to the small geometry holders used here) would result in 48 fA rms in a 5-kHz bandwidth.

Dielectric noise must also be expected to arise from the holder. The geometry of the holder and the dissipation factor of the dielectric material from which it is fabricated will determine this noise. Reported values of the dissipation factor of materials commonly used to fabricate patch pipette holders are variable and materials show variation between different manufacturers and production runs. The dissipation factor for polycarbonate is about 10^{-3}. Teflon has a lower dissipation factor, about 2×10^{-4}, but shows piezoelectric and space charge effects. Early measurements by the authors showed that either material could produce good holders with very similar noise. Polyethylene ($D \simeq 3\text{–}4 \times 10^{-4}$) and polypropylene ($D \simeq 3 \times 10^{-4}$) also produced low-noise holders. We have favored polycarbonate holders since this material is rigid and transparent and in actual practice has produced noise as low as that of any of the other materials mentioned. Lucite has a very high dissipation factor ($D \simeq 3\text{–}4 \times 10^{-2}$) and should not be used for holders when low noise is desired.

Any effort to calculate the expected dielectric noise of the holder will be rather imprecise both because of the degree of uncertainty regarding the dissipation factor of the material used and because of the rather complicated equivalent circuit of the holder. The headstage input is in series with the dielectric of the holder (polycarbonate, teflon, etc.) in part through direct contact and in part through air in the region where the wire extends through the holder but is not in contact with its walls. The outer surface of the holder is in turn capacitively coupled through air to its surroundings (e.g., grounded metal of headstage enclosure, microscope, chamber mount, manipulators, etc.). The analysis of dielectric noise from the series combination of different dielectrics is presented in greater detail below in regard to elastomer coating of patch pipettes. Here we will only describe the expected results for the holder. We have calculated that a small geometry (measured capacitance $\simeq$ 0.6 pF) polycarbonate holder will produce dielectric noise of about 15 fA rms in a 5-kHz bandwidth. A polycarbonate holder with a measured capacitance of ~1.5 pF should produce about 25 fA rms of dielectric noise in this bandwidth. Thus adding our small geometry holder to the headstage input should in total increase the 5-kHz noise (Axopatch meter) from 47 to 50 fA rms. A 1.5-pF polycarbonate holder is expected to increase this noise to 56 fA rms. These predictions are in good agreement with our measured results. The barrel of the holder can also be machined from quartz ($D \simeq 10^{-5}\text{–}10^{-4}$): a small holder with a quartz barrel and teflon end pieces might be expected to reduce the dielectric noise of the holder by more than a factor of two below that estimated for a small polycarbonate holder. Although the very low dissipation factor of quartz may make it an attractive material for this application as other noise sources continue to decline, at the present time further reductions of holder noise will not significantly improve the total noise of a patch clamp measurement.

Fabrication of quartz pipettes

Quartz softens at a temperature of over 1600°C, so no filament based puller is capable of pulling quartz patch pipettes. Sutter instruments' P-2000 puller uses a laser to generate sufficient heat to allow quartz to be pulled into patch pipettes. We have found that this puller, when used with quartz tubing having an outside diameter of 1.5 mm or less, is capable of reliably and repeatedly pulling patch pipettes from quartz. In our experience, its results become extremely variable when used with quartz tubing whose outside diameter exceeds 1.5 mm. If the outside diameter is kept to 1.5 mm or less, the puller works well even with extremely thick walls (i.e., OD/ID = 3.0). We have found that two stages of pull are best for producing patch pipettes. With a one stage pull, it is difficult to obtain a blunt taper and three stages generally results in increased thinning of the wall. We use the following program with the details of the tip geometry being modified only by changing the pull strength in the second stage. Stage 1: heat, 950; filament, 4; velocity, 30; delay pull, 150. Stage 2: heat, 950; filament, 4; velocity, 30 delay, 130; pull, 85–110. Higher settings for second stage pull produce a local thickening of the pipette wall near the tip. While we expect the detailed numbers to be different from one puller to another, we expect that the general approach exemplified in this program is likely to be successful in other instruments from the same company. When quartz is pulled, it almost immediately thins. The majority of this thinning occurs just beyond where the glass begins to taper. We found that with an initial OD/ID ratio of 1.43, an OD/ID ratio of about 1.2 was retained for the majority of the immersible part of the shank. For an initial OD/ID ratio of 2, we found that thinning led to a pulled OD/ID ratio of about 1.4 in the tapered region of the pipette. For OD/ID = 3 tubing with the same puller settings, a final OD/ID ratio of about 1.6 was maintained after pulling. Again, because of the high softening temperature of quartz, the kind of apparatus used to fire polish the tip of patch pipettes made from other glasses will not work with quartz. Fire polishing would require a laser or a flame, and so we chose to pull electrodes whose tip geometry is adequate to allow sealing to membranes without fire polishing. We have purchased quartz tubing from Garner Glass (Claremont, CA).

Elastomer coating

Despite the low dissipation factor and dielectric constant of quartz, we anticipated that coating the pipettes with a suitable elastomer such as Sylgard 184 would remain important in achieving low noise. Quartz patch pipettes can be coated with Sylgard or some other elastomer in exactly the same way as one coats any other patch electrode. As we will subsequently show, the best noise performance can be obtained if the elastomer coating extends all the way to the tip of the patch electrode. To do this, an electrode is placed in a specially designed syringe and holder (see Fig. 1 *a*) with the syringe barrel nearly fully withdrawn. Once the electrode is inserted, the syringe barrel is pushed inward as hard as possible to create an extremely strong positive pressure across the tip (7–8 atmospheres). The tip is immersed directly in the elastomer coating solution, withdrawn, and pointed skyward, still maintaining pressure, while curing the elastomer with a heat gun. This procedure can be repeated as many times as desired. Scanning electron micrographs (Fig. 1 *b*) show that

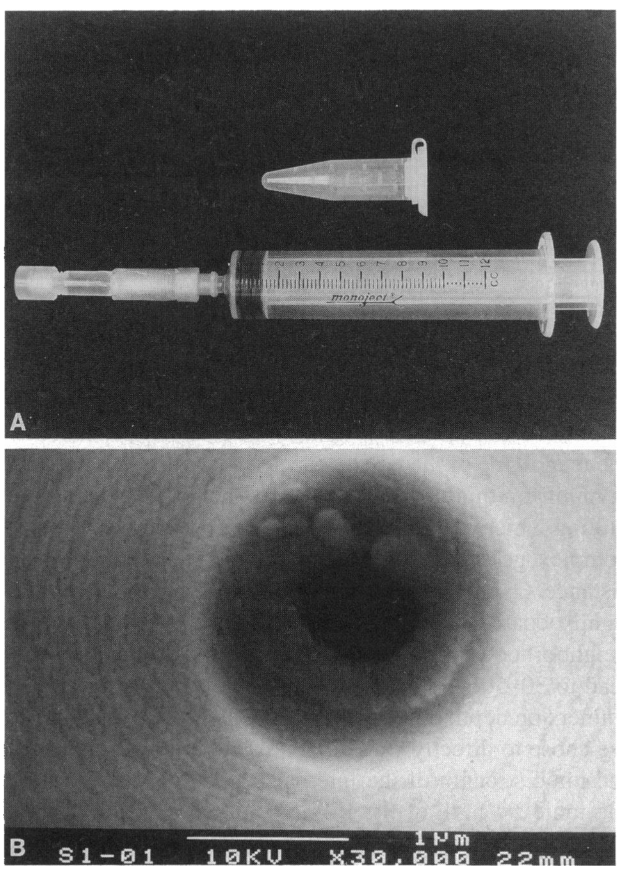

FIGURE 1 (*A*) Apparatus used for coating the electrode with Sylgard or other suitable elastomers to its tip. (*B*) Scanning electron micrograph of a quartz electrode tip which has been coated with Sylgard by the tip dip method described in the text.

the Sylgard extends to the tip and in some places coats a portion of the tip itself. Following the elastomer coating, the electrode can be placed in a fire polishing apparatus and the elastomer near the tip burned off if desired. This burning procedure made little difference in the ability of the electrodes to form gigohm seals at least with the three different cell types that we utilized (rabbit corneal epithelium, mouse and human lens epithelium). The electrodes sealed well (>80%) with or without burning Sylgard from the tip. It should be noted that when Sylgard is stored in a freezer after mixing, it is advisable to allow the container to be used to warm to room temperature prior to opening. The dissipation factor of Sylgard can apparently be increased by the absorption of water which may otherwise occur.

Several other elastomers can also be successfully used with this technique to produce noise performance that is comparable to that of Sylgard 184. We have found that GE RTV-165A and Dow Corning MDX4-1420 medical grade silastic both work quite well; the electrical characteristics of these elastomers are similar to Sylgard 184. Dow Corning R-6101 and Q1-4939 are reported by the manufacturer to have electrical properties that are superior to those of Sylgard, and therefore seem promising for low-noise applications. However, in our experience it is difficult to develop thick coats with Q1-4939; this elastomer also requires high heat for curing. To date we have found that R-6101 produces results at least as good as Sylgard, but our initial work has not demonstrated that it is clearly superior.

Tests of noise performance

When doing low-noise measurements, it is imperative that one systematically check the noise levels achieved at various stages of the experiment. The Axopatch 200A that we utilized for our measurements has a noise meter which reads the rms noise in a 5-kHz bandwidth through a four-pole Butterworth filter. Our particular Axopatch 200A has a noise level of 46 fA rms on the meter with nothing attached to the headstage input. With a holder in the headstage, the noise rises to 50 fA. With a quartz electrode filled with only enough solution to allow the reference electrode to be immersed and, with the electrode tip in air just above the bath, the noise level is typically 51–52 fA rms. On occasion, with the electrode tip in air, the noise may be significantly higher than this. In this situation we remove the electrode and check the noise of the holder itself. Most often, the noise of the holder is also elevated and the holder requires dismantling, sonication in 100% ethanol or distilled water, and drying in a 65°C oven for several hours. To keep the holder from becoming contaminated with fluid from the back of the electrode, it is important to maintain a suction line near the preparation attached to a hypodermic needle of just the right size to insert into the back of the pipette. The hypodermic needle and suction allows one to remove any excess fluid and routinely keeps fluid from getting into the holder. With care, holders can be used several weeks before they require cleaning.

Excess holder and pipette noise may also be related to the formation of films of fluid within the pipette and holder.

Patch pipette noise sources

The patch pipette contributes noise by several mechanisms. In the first place, simply adding the pipette to the holder (i.e., prior to immersion in the bathing solution) increases the capacitance at the headstage amplifier input by a small amount. This slightly increases noise by the mechanism already described for the holder. A small increase in dielectric noise is also expected.

Once the pipette is immersed in the bath and a gigohm seal has been formed, the pipette will also contribute noise due to the capacitance of the immersed portion of the pipette, C_e, in series with the input voltage noise, e_n, of the headstage amplifier. The mechanism is the same as discussed above and the PSD of this current noise component will rise as f^2. This noise, of course, is correlated with noise arising from e_n in series with other capacitances. C_e can range from as little as a small fraction of a pF for pipettes with a heavy elastomer coating and a shallow depth of immersion to 5 pF or more for uncoated or lightly coated pipettes with large immersion depths. Obviously this noise source increases as C_e increases, but for present day patch clamp amplifiers with low e_n it will be small in comparison with other noise sources described below regardless of the value of C_e. Aside from this "e_n-C_e" noise, the pipette contributes noise by at least four mechanisms in addition to the noise of the seal itself. These mechanisms (see Fig. 2), and how the noise attributed to them can be minimized, will now be described.

Thin film noise

Noise associated with films of solution which can form on the outside of an uncoated pipette as it emerges from the bathing solution (Fig. 2 *a*) can be very significant (Hamill et al., 1981). Such a film has a high distributed resistance in series with the distributed capacitance of the pipette wall. The power spectral density of this noise is expected to rise at low to moderate frequencies and then level out at higher frequencies (several kHz to several tens of kHz). Our attempts to parse this source of noise from total noise of uncoated pipettes sealed to Sylgard usually qualitatively show this behavior. With uncoated pipettes fabricated from Amersil T-08 quartz sealed to Sylgard (immersion depth $\simeq$ 2 mm), we have estimated that the noise associated with such films typically falls in the range of roughly 100–300 fA rms in a 5-kHz bandwidth. Interestingly, uncoated pipettes fabricated from GE quartz usually produce much less noise. In fact, such uncoated pipettes pulled from GE OD/ID = 2 tubing sealed to Sylgard with a ~2-mm depth of immersion have *total* noise as low as 100 fA rms in a 5-kHz bandwidth. Such a value is not much more than would be expected for such a pipette in the absence of any external solution film and may indicate that this quartz is relatively difficult to wet. With GE quartz, it appears that noise is elevated relative to coated pipettes primarily as the result of increased distributed RC noise (see below) resulting from the high capacitance of the uncoated pipette compared to coated pipettes, and so elastomer coating is still required.

Fortunately, coating the pipette with Sylgard 184 or other elastomers can essentially eliminate external film noise. The hydrophobic surface of suitable elastomers prevents the formation of such films. With coated quartz pipettes, total pipette noise can be accounted for without the need of invoking any contribution from this mechanism.

In principle, similar films of solution might form inside the pipette and/or in the holder. The noise contribution of such an internal film is expected to be less than that of the external films just considered, because the capacitance in series with an internal film should be less than that in series with an external film. This is because the portion of the pipette/holder involved in forming the capacitor in series with an internal film is not generally in contact with an external conductor, but instead is in contact with air. Even so, such an internal film could produce enough noise so that it should not be neglected. After filling the pipette with ionic solution, layering a few millimeters of silicone fluid or paraffin oil on top of the solution should prevent the formation of such an internal film. In our experience, however, this is not usually necessary if excess fluid is carefully removed from the pipette with suction as described previously.

Distributed RC noise

Although the majority of the resistance of the pipette is located near its tip, significant amounts of resistance reside in the shank region distal to the tip. Wall capacitance is distributed more or less evenly along the immersed portion of the pipette. The distributed resistance of the filling solution (i.e., its thermal voltage noise) in series with the distributed capacitance of the pipette wall will give rise to noise with a PSD that rises as f^2 over the range of frequencies important to patch clamping (DC to 100 kHz or more). We refer to this as *distributed RC noise* (Fig. 2 *b*). Theoretical predictions of the magnitude of the noise expected are imprecise due to the nonuniform thinning of pipette wall that often occurs during pulling which determines its capacitance, and the somewhat complex geometry of the pipette which determines its resistance. Oversimplified models suggest that for uncoated or lightly coated pipettes with "typical" OD/ID ratios in the neighborhood of 1.4, this mechanism would be expected to lead to 30–50 fA rms noise in a bandwidth of 5 kHz for an immersion depth of 2 mm. Rather than rely on such models, we chose to directly study this noise component by varying the ionic strength of the internal filling solution. Changing the ionic strength of the filling solution will not effect the pipette capacitance, but it will change the pipette resistance. For pipettes with equivalent geometry and the same depth of immersion, it is expected that in the frequency range studied the PSD of distributed RC noise will vary as $1/M$ and its rms value for a given bandwidth will vary as $1/M^{0.5}$, where M is the ionic concentration of the filling solution. In order to

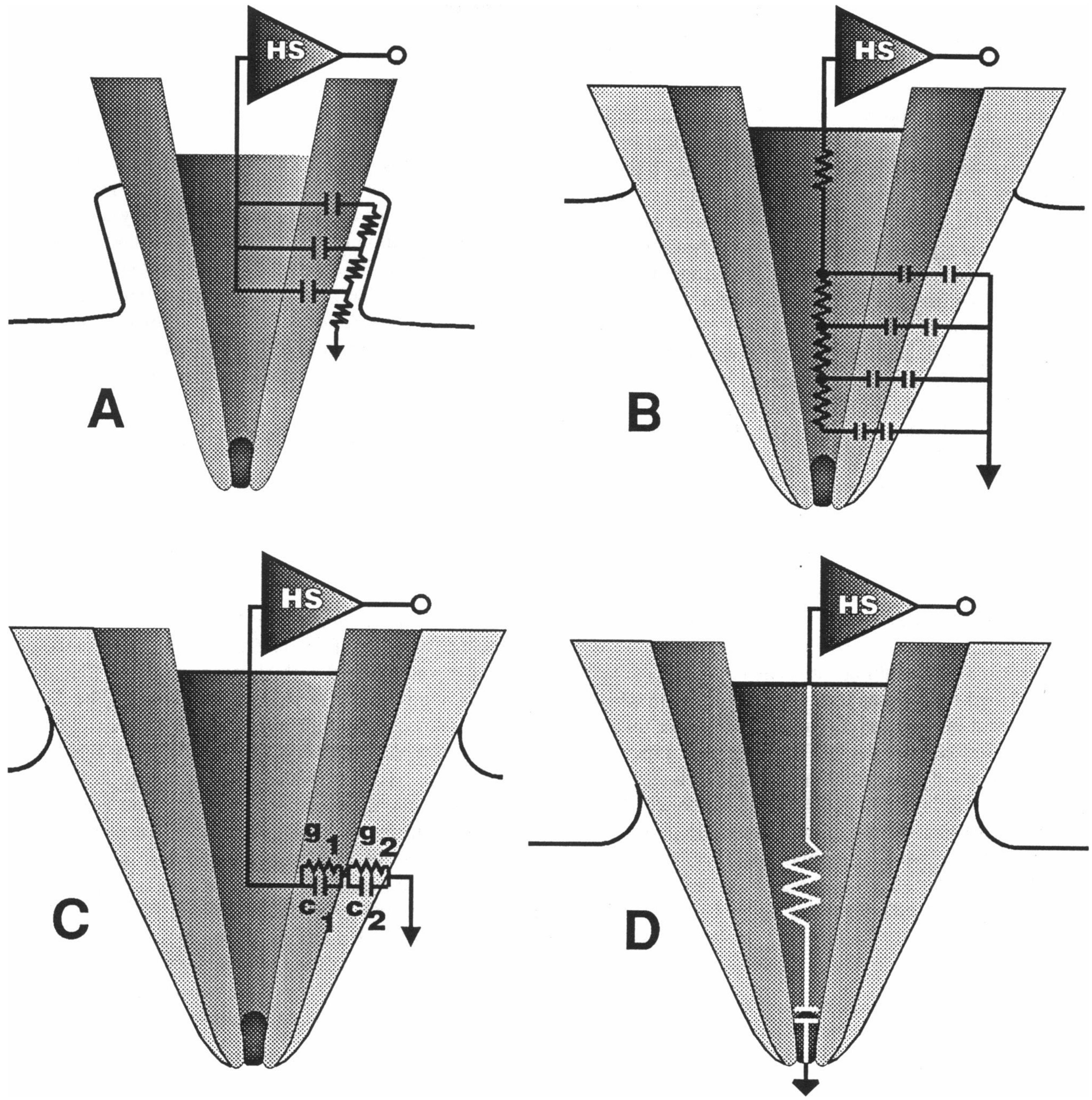

FIGURE 2 Simplified circuit representations of the major noise mechanisms of the patch pipette. (*A*) Thin solution film on the exterior surface of an uncoated patch pipette; noise arises from the thermal voltage noise of the distributed resistance of this film in series with the capacitance of the pipette wall. In *B*, *C*, and *D*, the pipette is shown coated with a suitable elastomer. (*B*) Distributed RC noise arising from the thermal voltage noise of the distributed resistance of the pipette filling solution in series with the distributed capacitance of the immersed portion of the pipette wall and its elastomer coating. (*C*) Dielectric noise of the series combination of the pipette (g_1, C_1, where $g_1 = \omega C_1 D_1$) and the elastomer coating (g_2, C_2, where $g_2 = \omega C_2 D_2$). (*D*) R_e-C_p noise arising from the thermal voltage noise of the entire (lumped) resistance, R_e, of the patch pipette in series with the patch capacitance, C_p. See text for further details.

quantify distributed RC noise, we used a chamber of about 1.8 mm in depth with a thin layer of Sylgard on its bottom. When an electrode tip is placed against Sylgard, it forms a seal that usually is too high in resistance to be measurable, certainly well in excess of 200 gigohms. We assume under these circumstances that the thermal noise due to the seal resistance is negligible and can be ignored. For distributed RC noise measurements, we paint the outer surface of the electrode with Sylgard only to the point where the electrode first begins to taper. This is very close to where the top of the bath will be when the electrode is sealed to Sylgard at the chamber bottom. This ensures that almost all of the electrode immersed in the bathing solution is not coated with Sylgard but that the thin film of fluid that would creep up the outer surface of the electrode is minimized by the Sylgard which extends just to the bath surface. Fig. 3 shows the power spectral densities recorded with several ionic strength filling solutions and with the electrode sealed to Sylgard. It is clear from the overplotted power spectral densities that the lower the ionic strength, the greater the noise. It should be noted that even if the pipette is filled with 1.5 M NaCl, the noise (73 fA rms in a 5-kHz bandwidth) is still not as low as is achieved for the same immersion depth with an extensively Sylgard-coated pipette filled with 150 mM NaCl and sealed to Sylgard (noise is typically less than 65 fA rms and has been as low as 57 fA rms in a 5-kHz bandwidth). From this it is apparent that one of the major benefits of elastomer coating is to decrease distributed RC noise by reducing the effective capacitance of the immersed portion of the pipette wall.

For 150 mM NaCl, the PSD of distributed RC noise for these uncoated pipettes (initial OD/ID = 2, 1.8-mm immersion depth) is approximated by $2.5 \times 10^{-38} f^2$ amp^2/Hz; the rms noise contribution is then $(8 \times 10^{-39} c_3 B^3)^{1/2}$ amps rms. For a bandwidth of 5 kHz, this indicates a noise component of about 35 fA rms for a four-pole Butterworth filter ($c_3 \simeq 1.2$). Thicker walled quartz, pulling techniques which thicken the wall near the tip, and/or heavy coating with an elastomer such as Sylgard can reduce the PSD of distributed RC noise by more than an order of magnitude. For example, for OD/ID = 3 quartz tubing with heavy Sylgard coating, we have achieved pipette capacitance, C_e, of ~0.35 pF for a 1.8-mm depth of immersion. This is four to five times less than the value achieved for OD/ID = 2 quartz pipettes that were not coated in the region immersed into the bath. For a constant depth of immersion and pipette resistance, the PSD of distributed RC noise is expected to vary in proportion to C_e^2. For a 2-mm depth of immersion for our pipettes, we believe that $\sim 10^{-14}\, C_e^2 f^2$ is a reasonable estimate of distributed RC noise PSD. Thus for $C_e \simeq 0.35$ pF its value should fall to about 10^{-39} amp^2/Hz. Rms noise in a 5-kHz

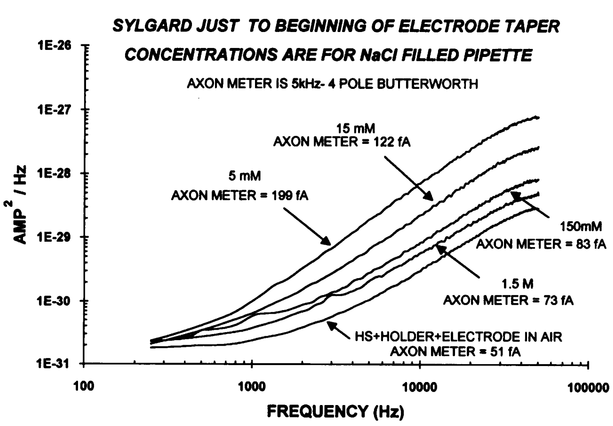

FIGURE 3 Power spectral density measurements of pipettes sealed to Sylgard at an immersion depth of 1.8 mm. As described in the text, the immersed portions of the pipettes were not coated with Sylgard. Ionic strength of the filling solution was varied from 5 mm to 1.5 M in an attempt to quantify distributed RC noise. The bend in the curves at high frequencies in this figure and in Fig. 5 is due to the presence of a four-pole Bessel filter with a −3-dB bandwidth of 50 kHz. See text for further details.

bandwidth due to this mechanism would be expected to fall to less than 10 fA. Shallow depths of pipette immersion can further reduce this noise component.

Dielectric noise

Dielectric noise (Fig. 2 *c*) has previously been a major, often dominant, source of noise arising from the pipette, since the dissipation factor of most glasses previously used for patch pipette fabrication is relatively high; e.g., 0.002 for Corning 7760, 0.003 for 7052, 0.005 for pyrex borosilicate, and 0.01 for soda lime glasses. The dissipation factor of quartz is much lower: $\sim 10^{-4}$ for Amersil T-08 and GE quartz to as little as $\sim 10^{-5}$ for Corning 7940. For a single dielectric with a dissipation factor D and a capacitance C_d, the dielectric noise PSD is given by

$$S_d^2 = 4kTDC_d(2\pi f) \quad \text{amp}^2/\text{Hz} \tag{5}$$

and rms noise in a bandwidth B is

$$i_d = (4kTDC_d c_2 \pi B^2)^{1/2} \quad \text{amp rms}, \tag{6}$$

where k is Boltzman's constant, T is absolute temperature, and c_2 is a coefficient which depends on the filter type (see Eq. 3). Thus, for example, 2 pF with $D = 10^{-4}$ would produce dielectric noise of about 17 fA rms in a 5-kHz bandwidth.

For pipettes coated with elastomers such as Sylgard 184, a more complicated equation which describes the dielectric noise of a series combination of two dielectrics with capacitances C_1 and C_2 and dissipation factors D_1 and D_2 is required. The basis for this equation is shown in Fig. 2 *c*. In this figure, the dielectric of the pipette wall and elastomer coating in the region immersed in the bath are represented as lumped ideal capacitances C_1 and C_2 in parallel with frequency-dependent loss conductances g_1 and g_2, defined by $g_1 = \omega C_1 D_1$ and $g_2 = \omega C_2 D_2$, where $\omega = 2\pi f$. Thus the admittance of each dielectric is simply $j\omega C_n + g_n$ ($n = 1, 2$) and the thermal noise PSD is given by $4kT$ multiplied by the real part of the admittance of the two series dielectrics. This thermal noise is what we call dielectric noise and in this case its PSD is given by

$$4kT(2\pi f)\frac{C_1D_1C_2D_2(C_1D_1 + C_2D_2) + D_1C_1C_2^2 + D_2C_2C_1^2}{(C_1D_1 + C_2D_2)^2 + (C_1 + C_2)^2} \quad \text{amp}^2/\text{Hz}. \tag{7}$$

For $D_1, D_2 \ll 1$ (as is the case here), this expression is well approximated by

$$4kT(2\pi f)\frac{D_1C_1C_2^2 + D_2C_2C_1^2}{(C_1 + C_2)^2} \quad \text{amp}^2/\text{Hz}. \tag{8}$$

Using Eq. 8, the rms noise in a bandwidth B is then given by

$$\left[4kTc_2\pi B^2\frac{D_1C_1C_2^2 + D_2C_2C_1^2}{(C_1 + C_2)^2}\right]^{1/2} \quad \text{amps rms}. \tag{9}$$

We consider C_1 and D_1 to be the capacitance and dissipation factor of the quartz pipette and C_2 and D_2 to be the capacitance and dissipation factor of the elastomer coating.

The capacitance C_1 of Eqs. 7, 8, and 9 depends on the wall thickness of the immersed portion of the pipette and on the dielectric constant of the glass. Since the dielectric constant of quartz is 3.8, it can be predicted that for a 2-mm immersion depth that for OD/ID = 1.2 (after pulling), C_1 = 2.3 pF, for OD/ID = 1.4 (after pulling), C_1 = 1.2 pF, and for OD/ID = 1.6 (after pulling), C_1 = 0.9 pF. Measured values for pipettes without elastomer coating in the immersed region are usually somewhat higher, e.g., about 1.6 pF for tubing with an initial OD/ID ratio of 2 (~1.4 after pulling) and an immersion depth of about 2 mm. This may be due to a meniscus of fluid which increases the actual effective depth of immersion and/or to nonuniformities in wall thickness.

The capacitance C_2 of the elastomer coating will, of course, decrease as the thickness of the coating increases. With the usual methods of elastomer coating, it is difficult to achieve thick coats very near the pipette tip. The dip method of coating described above can build up heavier coats all the way to the tip, but the thickness of the coat remains nonuniform. We have therefore preferred to estimate values of C_2 by measuring the actual capacitance, C_e, of a coated pipette with a known immersion depth and assume that C_2 can be calculated from this information and estimates of C_1 such as those described above, i.e., $C_2 = C_1C_e/(C_1 - C_e)$. For a 2-mm immersion depth with heavy Sylgard coating, the lowest practical value of C_e we have achieved is ~0.35 pF with pipettes drawn from OD/ID = 2 quartz tubing. Assuming that $C_1 \simeq 1.6$ pF, this indicates that $C_2 \simeq 0.45$ pF. Heavier coatings are possible, but the pipettes become more difficult to work with. For light coats of Sylgard, the value of C_2 can easily be as much as 2 pF or more.

The dissipation factor of the elastomer is very important to its noise performance. As already noted, the dissipation factor of quartz is in the range of 10^{-5} to 10^{-4}; for the quartz with which we have worked to date, 10^{-4} is a reasonable estimate. The dissipation factor of Sylgard 184 is reported to be in the range of 0.0009–0.002, and we believe that 0.002 is a reasonable estimate. Because the dissipation factor of Sylgard is much higher than that of quartz, the series combination of a quartz pipette and a Sylgard coating (with realistic values of C_2) will actually produce *more* dielectric noise than would be produced by the pipette alone, despite the reduced overall capacitance. This can be appreciated from Fig. 4 *a*.

Fig. 4 shows plots of rms dielectric noise in a 5-kHz bandwidth predicted on the basis of Eq. 9. In both Fig. 4 *a* and 4 *b* the capacitance of the elastomer coating (C_2) varies from 0.1 to 10 pF, which somewhat exceeds the practical range of this capacitance. Several values of C_1 (i.e., the capacitance of the quartz pipette itself) are shown in each panel. These can be interpreted as corresponding to different OD/ID ratios (for a fixed depth of immersion) or to different depths of immersion. For example, with pipettes pulled from OD/ID = 2 quartz tubing, C_1 = 4 pF is expected to correspond to an immersion depth of 4–5 mm; C_1 = 1 pF would correspond to roughly 1 mm of immersion. The dissipation factor of quartz (D_1) is taken to be 10^{-4} in both Fig. 4 *a* and 4 *b*. In

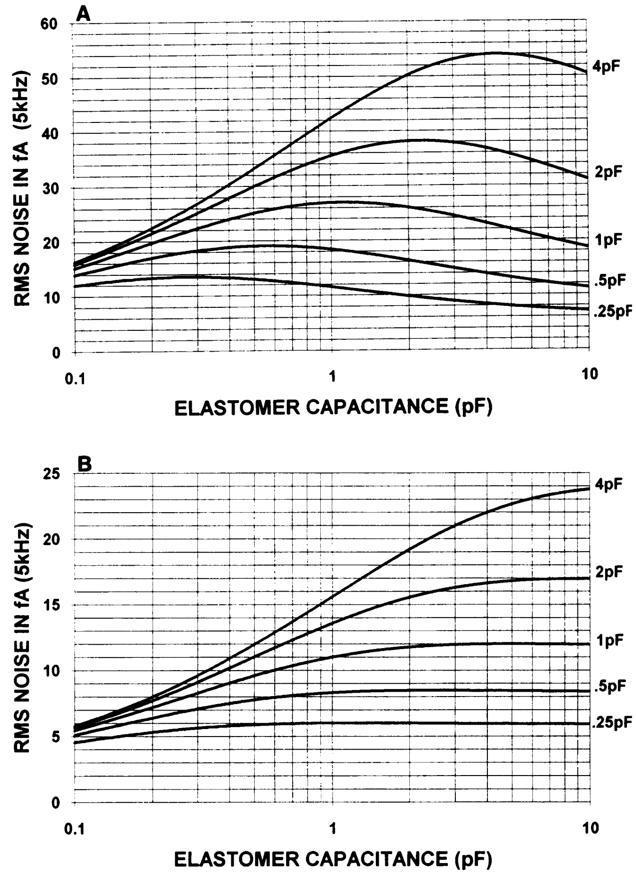

FIGURE 4 rms dielectric noise predicted by Eq. 9 for elastomer-coated quartz pipettes in a 5-kHz bandwidth. Noise is plotted as a function of the capacitance of the elastomer coating (C_2) for five different values (0.25, 0.5, 1, 2, and 4 pF as labeled at the right) of the capacitance of the immersed portion of the pipette itself (C_1 in Eq. 9). In both panels the dissipation factor, D_1, of the quartz is 10^{-4}. In *A* the dissipation factor of the elastomer, D_2, is 0.002; in *B*, D_2 = 0.00025. See text for further details.

4 *a* the dissipation factor of the elastomer coating (D_2) is 0.002, which approximates Sylgard 184. With this value of D_2, it can be seen that the predicted dielectric noise actually peaks. For C_1 = 4 pF, this peak occurs at $C_2 \simeq 4.5$ pF. For lower values of C_1 the peak occurs at correspondingly lower values of C_2. The C_2 = 2 pF curve in 4 *a* corresponds to a pipette pulled from OD/ID = 2 quartz tubing with a depth of immersion of about 2 mm or somewhat more. For this curve, an elastomer capacitance (C_2) of 2 pF would correspond to a light coat of Sylgard; C_2 = 1 pF roughly corresponds to a moderate Sylgard coating, and C_2 = 0.5 pF corresponds to a heavy coat. From the figure, dielectric noise decreases from 38 to 36 to 30 fA rms for these values of C_2. In this case, heavier coats of Sylgard somewhat decrease the dielectric noise. However, it must be realized that the same quartz pipette *without* Sylgard would only have produced about 17 fA rms noise (Eq. 6 with $D = 10^{-4}$ and C_d = 2 pF). It is worth noting that a lower dissipation factor quartz (e.g., Corning 7940 with $D \simeq 10^{-5}$, not shown) will have little effect on dielectric noise once the pipette is coated with Sylgard. Thus for C_1 = 2 pF, $D_1 = 10^{-5}$, and values of C_2 in

the range considered above (0.5–2 pF) with $D_2 = 0.002$, equation 9 predicts that the dielectric noise of the pipette will be only about 1% less than for $D_1 = 10^{-4}$.

Clearly, coating a quartz pipette with Sylgard 184 is not advantageous from the point of view of dielectric noise. However, coating with Sylgard or some other suitable elastomer is required to eliminate noise associated with films of solution that can otherwise form on the exterior surface of the pipette, and to minimize distributed RC noise. The net effect is that heavily Sylgard-coated quartz pipettes display the lowest noise. This becomes more apparent as bandwidth increases since the rms value of dielectric noise increases linearly with increasing bandwidth, while that of distributed RC noise increases as $B^{1.5}$, making distributed RC noise relatively more important as bandwidth increases.

Although Sylgard coating can actually increase the dielectric noise associated with a quartz pipette, an elastomer with a lower dissipation factor could be effective in decreasing total dielectric noise. This can be seen in Fig. 4 *b*. The format of this figure is the same as in 4 *a* (but note change of vertical scale) and the parameters are the same, except that in 4 *b* the dissipation factor of the elastomer coating (D_2) is assumed to be 2.5×10^{-4}. This value corresponds to the dissipation factor claimed by the manufacturer for Dow Corning R-6101 and Q1-4939. In this case, the peak in the curves described in Fig. 4 *a* is imperceptible and dielectric noise declines smoothly from a maximum plateau for high values of C_2 (corresponding to very light elastomer coating) to lower values as the elastomer coating becomes thicker (i.e., C_2 decreases). In this case the plateau value at high values of C_2 corresponds to the dielectric noise of the quartz pipette itself, i.e., without elastomer coating. It is therefore obvious that coating a quartz pipette with such an elastomer will decrease the dielectric noise of the pipette, with heavier coatings producing progressively smaller noise. Unfortunately, however, our preliminary measurements of pipettes coated with R-6101 have not demonstrated that it is significantly superior to Sylgard 184, although it is certainly not worse. At present, we are unable to account for these preliminary results, although we suspect that the dissipation factor of this elastomer may be higher than the value claimed by the manufacturer.

Fig. 4 can also be used to judge the effects of immersion depth on dielectric noise. Consider a moderately Sylgard coated quartz pipette pulled from OD/ID = 2 tubing. For an immersion depth of about 2 mm this is approximated by $C_1 = 2$ pF and $C_2 = 1$ pF; from Fig. 4 *a* the predicted dielectric noise is about 36 fA rms in a 5-kHz bandwidth. Assuming uniform OD/ID ratios for the quartz pipette and the Sylgard coating, reducing the immersion depth to about 1 mm would correspond to $C_1 = 1$ pF and $C_2 = 0.5$ pF, and it is predicted that dielectric noise is reduced to about 25 fA rms. For a 0.5-mm immersion depth ($C_1 = 0.5$ pF, $C_2 = 0.25$ pF), dielectric noise further decreases to about 18 fA rms. However, with very heavy elastomer coating the meniscus of solution surrounding the pipette as it emerges from the bath may be significant. Because of this, withdrawing the pipette tip toward the surface with an excised patch or reducing the bath level for an on-cell patch sometimes does not decrease the pipette capacitance, or pipette noise, as much as expected.

Our estimates of dielectric noise from Sylgard-coated pipettes sealed to Sylgard, as well as from actual cell patches, are in good agreement with the predictions given above. An example is presented in Fig. 5, which shows the over-plotted power spectral densities of an electrode whose tip is in air just above the bath and the same electrode when sealed to Sylgard at the bottom of the 1.8-mm deep chamber. The additional noise should be dominated by dielectric noise. This is verified by doing the difference spectrum between the two as shown in the figure. The difference spectrum which is a good approximation of the noise of the pipette, can be fit by $8 \times 10^{-35}f + 4.5 \times 10^{-39}f^2$ amp²/Hz. Its rms noise contribution is then $(4 \times 10^{-35}c_2B^2 + 1.5 \times 10^{-39}c_3B^3)^{1/2}$ amps rms. The measured capacitance of this pipette, C_e, was 0.65 pF. As described above, using $C_1 = 1.6$ pF, we estimate that C_2 (i.e., the capacitance of the Sylgard coating) is 1.1 pF. Using Eq. 7 with these parameters (and $D_1 = 0.0001$, $D_2 = 0.002$) produces an estimate of dielectric noise PSD that is essentially identical to the fitted value (i.e., $8 \times 10^{-35}f$ amp² Hz). The rms value of the dielectric noise for this pipette is then estimated to be about 33 fA rms in a 5-kHz bandwidth. On the basis of the previous discussion associated with Fig. 3, distributed RC noise PSD for this pipette is predicted to be about $4.1 \times 10^{-39}f^2$, again very close to the fitted value; here, however, the actual agreement is probably not quite as good as it seems, since the difference spectrum also contains a small amount of f^2 noise arising from the pipette capacitance and e_n.

Fig. 5 is representative of our measurements of dielectric noise for Sylgard coated thick-walled quartz pipettes sealed

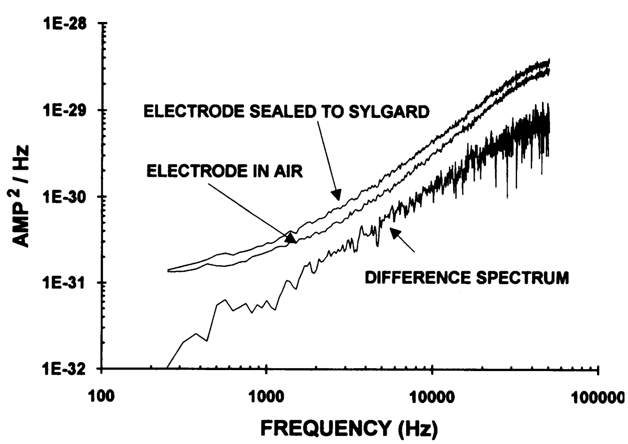

FIGURE 5 Power spectral density of the total patch clamp noise for a typical quartz pipette sealed to Sylgard ($R_{sh} > 200$ GΩ) at a depth immersion of approximately 1.8 mm, and the same pipette in air just above the bathing solution. The pipette was pulled from OD/ID = 2 quartz tubing and coated to the tip with Sylgard 184; measured pipette capacitance for the Sylgard-sealed curve was ~0.65 pF. The lower (noisy) curve is the difference between these two power spectral densities and represents the PSD of the noise contributed by the immersed (and sealed) pipette. The Axopatch 200A noise meter reading for the pipette sealed to Sylgard was 64 fA rms. See text for further details.

to Sylgard at a depth of immersion of about 2 mm; estimates generally fall in the range of 20–35 fA rms in a 5-kHz bandwidth. In actual excised patch measurements with the pipette tip withdrawn toward the surface of the bath, estimates of dielectric noise have sometimes been less than 15 fA rms in a 5-kHz bandwidth. For comparison, our previous measurements of light to moderately Sylgard coated pipettes fabricated from more than 20 types of glass other than quartz (Rae and Levis, 1984a, 1992a) indicated that for a 2-mm immersion depth, pipette dielectric noise was not less than ~100 fA rms in a 5-kHz bandwidth for the best glasses tested, and could be as much as ~200 fA rms in this bandwidth. On the basis of Eq. 9, somewhat lower values of dielectric noise are predicted for such glasses with heavy Sylgard coatings. More recent measurements with pipettes fabricated from Corning 7052 and 7760 (which is no longer available) that were heavily Sylgard-coated to the tip by means of the dipping technique described above, indicate that dielectric noise as low as ~70 fA rms can be achieved in a 5-kHz bandwidth for a 2-mm depth of immersion. This is still more than twice the dielectric noise typical of a Sylgard-coated quartz pipette. If otherwise suitable elastomers can be found with dissipation factors lower than Sylgard 184, they will be expected to reduce the noise of both quartz pipettes and pipettes fabricated from other types of glass, but a relative advantage of quartz would remain.

R_e-C_p noise

The final noise mechanism associated with the pipette arises from the thermal voltage noise of the entire pipette resistance, R_e, in series with the patch capacitance, C_p (Fig. 2 *d*). Up to frequencies of about $1/2\pi R_e C_p$, this noise is expected to have a PSD given by

$$S_{ep}^2 = 4\pi^2 e_e^2 C_p^2 f^2 \quad \text{amp}^2/\text{Hz}, \tag{10}$$

where $e_e{}^2 = 4kTR_e$. Only thermal voltage noise is assumed for the pipette resistance; low frequency excess noise has been neglected since the currents through the pipette are expected to be very small. The rms value of this noise component in a bandwidth B is

$$i_{ep} = \{1.33\pi^2 c_3 e_e^2 C_p^2 B^3\}^{1/2} \quad \text{amps rms}. \tag{11}$$

According to the measurements of Sakmann and Neher, 1983, patch capacitance may be expected to fall in the range of 0.01 to ~0.25 pF for pipette resistances in the range of 1 to 10 MΩ. Although their measurements contained a great deal of scatter, as expected, high patch capacitances were correlated with lower pipette resistances. On the basis of Eqs. 10 and 11 it is expected that the lowest noise will be associated with small patches, even if this requires somewhat higher pipette resistances. Thus for a very unfavorable example in terms of noise with R_e = 2 MΩ and C_p = 0.25 pF, this "R_e-C_p" noise is predicted to be 63 fA rms in a 5-kHz bandwidth. However, in a very favorable situation with R_e = 10 MΩ and C_p = 0.01 pF, it is expected to be only about 6 fA rms in this bandwidth.

Seal noise

The current noise power spectral density of the membrane-glass seal for zero applied voltage is expected to be given by $4kT\,Re\{Y_{sh}\}$, where $Re\{Y_{sh}\}$ is the real part of the seal admittance. Low frequency excess noise is also possible when current crosses the seal resistance. The minimum value of $Re\{Y_{sh}\}$ is $1/R_{sh}$, where R_{sh} is the DC seal resistance. In actual practice, what is usually measured as "seal" resistance, R_{sh}, will be the parallel combination of the resistance of the seal and of the patch membrane (with all known channels closed). However, unless the patch membrane is damaged, we expect that its resistance will usually be high in comparison with that of the seal. A minimum estimate of the rms noise contribution from the seal is $(4kTc_1B/R_{sh})^{1/2}$, i.e., simply the thermal current noise of the seal resistance. While it is certainly possible that seal noise sometimes exceeds this minimum estimate, our measurements from several patches with R_{sh} in the range of 40–100 GΩ indicate that the noise of the seal is often indistinguishable from the expected thermal current noise of the seal resistance (Rae and Levis, 1992b). Thus in a 5-kHz bandwidth, seal noise may be as low as 90, 40, and 20 fA rms for seal resistances of 10, 50, and 200 GΩ, respectively. The noise of a high resistance seal can thus be small in comparison to other noise sources described above—except, perhaps, at very low bandwidths. As bandwidth increases, the thermal current noise of the seal will become relatively less important since its rms contribution only increases as $B^{0.5}$, while that of dielectric noise increases as $B^{1.0}$, and that of distributed RC noise and R_e-C_p noise increase as $B^{1.5}$.

Noise with real cell patches

While working on the quartz technology, we routinely kept track of the rms noise in 5 kHz with a quartz electrode sealed to our cells. In addition, we routinely measured the seal resistance. Fig. 6 is a scatter plot of the noise of every membrane patch achieved during the time of our study plotted against the seal resistance achieved. Several points are obvious from this graph. The first is that even under the worst

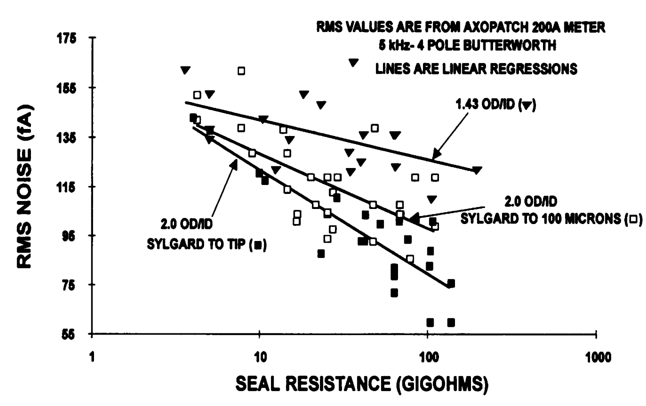

FIGURE 6 Scattergram of the rms noise in a 5-kHz bandwidth (four-pole Butterworth filter) with quartz electrodes sealed to real cells plotted against the seal resistance achieved.

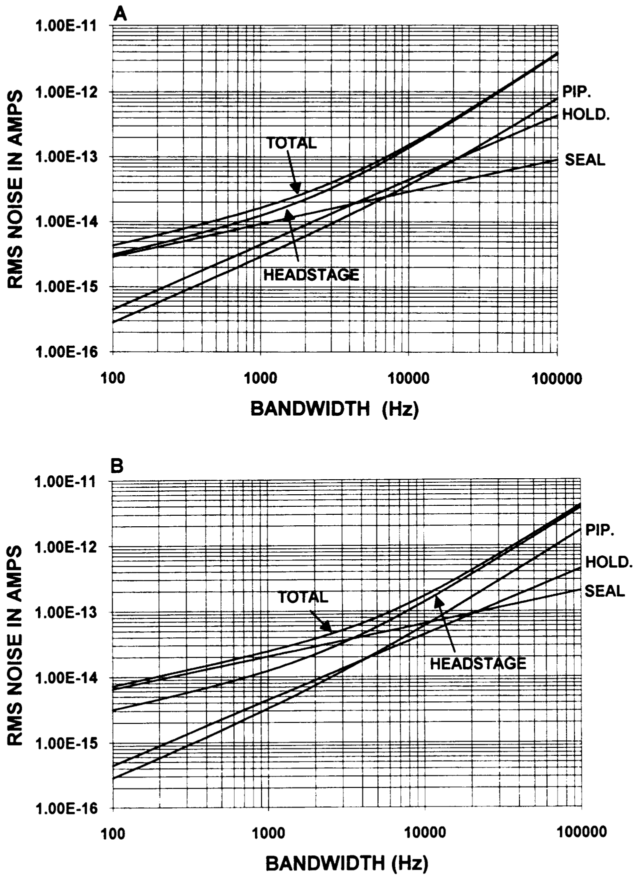

FIGURE 7 Predicted values of the rms noise as a function of bandwidth (−3 dB, eight-pole Bessel filter, 100 Hz to 100 kHz) for a "best-case" (*A*) and "typical" (*B*) measurement situation with elastomer-coated quartz pipettes. In both panels individual noise components illustrated include the seal, holder (labeled *HOLD.*), pipette (labeled *PIP.*), and headstage. The rms sum of these noise sources is labeled as "*TOTAL*" in each panel. In the case of the seal, only thermal current noise is assumed. The holder noise only represents dielectric noise of the holder. The pipette noise curve represents the sum of distributed RC noise, dielectric noise, and R_e-C_p noise. Headstage noise includes contributions from e_n in series with holder and pipette capacitance. In *A*, the following parameters have been assumed: R_{sh} = 200 GΩ. Holder dielectric noise PSD is characterized by $1.5 \times 10^{-35} f$ amp²/Hz. The pipette is characterized by R_e = 8 MΩ and C_p = 0.01 pF; the PSD of pipette distributed RC noise is given by $4 \times 10^{-40} f^2$ amp²/Hz, and the PSD of pipette dielectric noise is given by $1.2 \times 10^{-35} f$ amp²/Hz. These parameters produce pipette noise that approximates our best results to date, and are reasonable for a pipette pulled from quartz ($D_1 = 10^{-4}$) tubing with OD/ID > 2 with a heavy elastomer coating (D_2 = 0.001 − 0.002) and a shallow depth of immersion of about 0.5 mm ($C_e \simeq 0.2$ pF). In *B*, the seal resistance, R_{sh}, is 40 GΩ, which is roughly the average value for the cells we have used. The dielectric noise PSD of the holder is given by $4 \times 10^{-35} f$ amp²/Hz. The pipette is characterized by R_e = 4 MΩ and C_p = 0.04 pF; the PSD of distributed RC noise is given by $8 \times 10^{-39} f^2$ amp²/Hz; and the PSD of pipette dielectric noise is given by $10^{-34} f$ amp²/Hz. These parameters are reasonable for a quartz pipette pulled from OD/ID ≤ 2 tubing with a moderate elastomer coating and a depth of immersion of about 2 mm ($C_e \simeq 1$ pF).

circumstances where the seal resistances are only a few gigohms, the background noise with quartz pipettes is almost always less than 150 fA in a 5-kHz bandwidth. Early attempts using quartz pipettes with an initial OD/ID ratio of 1.43 usually produced noise levels worse than quartz pipettes pulled from OD/ID = 2 tubing similarly Sylgard-coated to about 100 μm back from the tip. Coating the electrodes with Sylgard all the way to the tip produced an even further improvement in the noise so that seal resistances in excess of 20 gigohms rather routinely resulted in noise at the bottom of the chamber (1.8-mm immersion depth) less than 100 fA. Of the measurements shown, those using pipettes Sylgard-coated to the tip and pulled from OD/ID = 2.0 quartz tubing (*filled squares*) are probably the most representative of possible performance. These were the most recent measurements made and are the most consistent in terms of technique and in terms of the amount of elastomer coating. For about half of these patches, noise is well accounted for by our predictions for pipette noise plus the expected thermal current noise of the seal. The best noise achieved was 60 fA rms in a 5-kHz bandwidth.

DISCUSSION

These studies allow us to draw several conclusions concerning low noise patch clamp measurements:

1. Quartz pipettes allow substantially lower noise patch clamp recordings than pipettes made from other glasses.

2. The largest OD/ID ratio tubing produce the lowest noise pipettes. If methods can be found to more closely persevere the initial OD/ID ratio as the tubing is drawn out into pipettes, further improvements are expected.

3. Elastomer coating is still required even for quartz pipettes. In fact, heavy coating all the way to the tip produces the lowest noise. Coating with an appropriate elastomer prevents the formation of thin films of solution, and the noise they produce, on the outside of the pipette. Heavy elastomer coating also decreases the capacitance of the pipette by thickening its wall; the major benefit of this is the reduction of distributed RC noise.

4. For pipettes of equivalent geometry, the only characteristic of the glass per se that is important to distributed RC noise is its dielectric constant. Thus this noise is not expected to depend strongly on the type of glass. Distributed RC noise is minimized by thick walled pipettes and heavy elastomer coating to the tip of the pipette. Shallow depths of immersion should also reduce this noise.

5. Dielectric noise of quartz pipettes is significantly less than that of pipettes fabricated from other types of glass, due to the extremely low dissipation factor of quartz ($D \simeq 10^{-4}$). Coating with Sylgard 184 ($D \simeq 2 \times 10^{-3}$) will actually somewhat increase the dielectric noise of quartz pipettes, although heavily Sylgard-coated quartz pipettes remain superior to pipettes fabricated from other glasses. Elastomers with lower dissipation factors than Sylgard 184 would further reduce dielectric noise. Dielectric noise is also minimized by shallow depths of immersion.

6. With pipettes fabricated from 1.5-mm OD/0.75-mm ID quartz tubing, on-cell or inside-out patches of real membranes will almost always have rms noise levels at 5 kHz (four-pole Butterworth) of <150 fA at the bottom of a

1.8-mm saline-filled chamber even for seal resistances less than 10 gigohms. With Sylgard coating to the tip and seal resistances >20 gigohms, noise is usually <100 fA and has been as low as 60 fA rms.

7. For quartz pipettes, the use of heavy elastomer coating to the tip of the pipette can replace the use of the silicone (dimethylpolysiloxane) fluid technique which we have described previously (Rae and Levis, 1992b).

Fig. 7 summarizes "best-case" and "typical" noise performance for single channel patch clamp measurements using the best present-day electronics and Sylgard-coated quartz pipettes. In both panels, the rms value of total noise and of the major noise components are plotted as a function of bandwidth from 100 Hz to 100 kHz; Fig. 7 *A* represents "best-case" and Fig. 7 *B* illustrates "typical" noise performance. Here an eight-pole Bessel filter has been assumed to more accurately reflect the noise observed in time-domain channel measurements. The curves are theoretical, but closely reflect actual measured performance. In each case, the seal has been assumed to produce only thermal noise. The best-case predictions assume a 200-GΩ seal, a shallow depth of immersion, heavy Sylgard coating and a small patch capacitance; the typical performance curves assume a 40-GΩ seal, a ~2-mm depth of immersion, somewhat lighter Sylgard coating, and a moderate patch capacitance. In both cases, the headstage noise illustrated includes correlated noise components arising from e_n in series with the holder and pipette capacitance, and therefore is somewhat higher than the open-circuit noise of the headstage amplifier. Only dielectric noise is assumed for the holder. Further details of the assumed parameters are listed in the figure legend. In a 5-kHz bandwidth the "best-case" noise is 65 fA rms and the "typical" noise is 95 fA rms.

In the best-case situation, the noise of the pipette is significantly less than that of the headstage. In fact, pipette noise is less than the noise of the holder up to bandwidths of about 20 kHz. It should be noted that estimates of pipette noise from our best results for both pipettes sealed to Sylgard and real excised patches are at least as good as the prediction shown in this figure. Pipette noise is assumed to arises from distributed RC noise, dielectric noise, and R_e-C_p noise. It is interesting to note that at very wide bandwidths (above about 50 kHz) in this example where distributed RC and dielectric noise are minimized, R_e-C_p noise is the largest component of pipette noise—even though this example uses a very small patch capacitance (0.01 pF). At bandwidths below about 500 Hz, the headstage and the seal (200 GΩ) contribute about equal amounts of noise. At wider bandwidths in the best-case situation, it is obvious that the dominant source of noise is the headstage amplifier itself. The rms value of pipette noise is never more than about one fourth of that of that of the headstage; indeed, the noise of the pipette remains below 100 fA rms at a bandwidth of 20 kHz.

In the "typical" performance curves, it can be seen that the seal is the dominant noise source up to about a bandwidth of 4 kHz. At higher bandwidths, the pipette and the headstage contribute similar amounts of noise, although pipette noise remains below that of the headstage amplifier.

Although it is likely that refinements of patch pipette technology may increase the percentage of patches which more closely approximate the best-case than the typical predictions just given, it seems evident that further improvements in total noise performance will require lower noise electronics. In the past, it has sometimes been argued that improvement in electronic noise was of little benefit to overall patch clamp noise since the pipette and the seal were assumed to dominate total noise. With quartz pipettes and the techniques we have described this is clearly not the case. In fact, comparison of the best case total noise with the sum of seal noise and best case pipette noise (recalling that holder noise can be further reduced if the effort seems worthwhile) indicates that the potential presently exists by means of improved electronics to reduce noise below the best results achieved to date by as much as a factor of more than 2 at a bandwidth of 5 kHz, a factor of 3 at a bandwidth of 10 kHz, and nearly a factor of 4 at a bandwidth of 30 kHz.

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REFERENCES

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York and London. 191–263.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.* 391:85–100.

Levis, R. A., and J. L. Rae. 1992a. Constructing a patch clamp setup. *Methods Enzymol.* 207:18–66.

Rae, J. L., and R. A. Levis. 1984a. Patch voltage clamp of lens epithelial cells: theory and practice. *Mol. Physiol.* 6:115–162.

Rae, J. L., and R. A. Levis. 1984b. Patch clamp recordings from the epithelium of the lens obtained using glasses selected for low noise and improved sealing properties. *Biophys. J.* 45:144–146.

Rae, J. L., and R. A. Levis. 1992a. Glass technology for patch electrodes. *Methods Enzymol.* 207:66–92.

Rae, J. L., and R. A. 1992b. A method for exceptionally low noise single channel recordings. *Pflugers Arch.* 42:618–620.

Rae, J. L., and R. A. Levis. 1993. Quartz pipettes for single channel recording. *Biophys. J.* 64(2, pt. 2):A201.

Sakmann, B., and E. Neher. 1983. Geometric parameters of pipettes and membrane patches. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York and London. 37–51.

Sigworth, F. J. 1983. Electronic design of the patch clamp. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York and London. 3–35.