Pflügers Arch (1992) 420:618–620

Pflügers Archiv
European Journal of Physiology

Short communication

A method for exceptionally low noise single channel recordings

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Received March 2, 1992/Accepted March 17, 1992

Summary

We present a method whereby, with integrating electronics, quartz patch electrodes and a novel use of silicone oil, background noise levels as low as .083 pA RMS in a 5 kHz bandwidth (4-pole Butterworth filter) have been achieved in single channel patch clamp recordings. These approaches result in much higher signal to noise ratios for single channel recording than have previously been reported and should allow many investigators to significantly reduce noise at a constant bandwidth or to increase their recording bandwidths by several kHz.

Key words: patch clamp, quartz, integrating electronics, single channels, silicone oil.

Introduction

The present levels of background noise in single channel recordings has limited investigators to recording at bandwidths of 1 kHz or less for small conductance channels and to bandwidths not much greater than 10 kHz even for channels with a large single channel conductance. The noise in single channel recordings is contributed by the electronics, the electrode holder, the electrode glass, and the gigohm seal to the membrane. Noise components sum as root mean square (RMS) quantities meaning that the resultant total noise is equal to the square root of the sum of the squared individual RMS components contributing to the noise. In measurements to date with high resistance seals, it has been true approximately that 1/2 of the residual noise comes from the electronics and 1/2 from the holder and electrode. In order to improve the overall noise in a patch clamp recording, it is necessary to reduce the noise from each of the major noise contributing components.

Electronic Noise

Recently, major improvements in electronic noise have been made through use of integrating technology where a capacitor rather than a gigohm valued resistor is the feedback element in the current to voltage converter. Using this technology, amplifier noise as low as .08 pA RMS in a 5 kHz bandwidth (4-pole Butterworth filter) has become routinely possible. Here we use an Axopatch 200A (Axon Instruments, Foster City, CA) which has a noise level of .057 pA RMS in a 5 kHz bandwidth.

Quartz Technology

We use quartz electrodes made from Amersil T-08 quartz drawn into capillary tubing of 1.65 mm outside diameter - 1.15 mm inside diameter by Garner glass (Claremont, CA). The tubing was pulled into patch electrodes using a Sutter Instruments (Novato, CA) Model P-2000 laser based puller using a two stage pull. The resulting patch electrodes had tip resistances between 6 and 10 MΩ and shanks about 10 mm in length. We have not found it possible, to date, to pull this particular quartz tubing into tips which are blunt and yet small enough to allow gigohm seals to form routinely without fire polishing. Fire polishing of quartz electrode tips is difficult since, due to the 1600° C softening point of quartz, it must be done in a flame. Consistent fire polishing by this approach has been hard to achieve and so we opted to pull smaller tips than we use normally for patch clamping since these tips would routinely seal to our cells without fire polishing. Even with the low dissipation factor of quartz electrodes (3,5), it is necessary to coat their outside surface with Sylgard #184 (Dow Corning, Midland, MI) to keep a noise producing thin film of saline from creeping up the side of the electrode once the tip is immersed in the bath (2). Because the tips were sharp, relatively thin, and somewhat brittle, it was not routinely possible to apply the Sylgard closer than 100 microns from the tip. Because of the possibility that a thin film of Sylgard might coat the tip, we heated the tips with a conventional firepolisher to remove this film.

Measurements from Cornea Cells

The electrode tips were pushed against rabbit corneal epithelial cells under direct observation and gentle suction was applied until a gigohm seal formed (4). With the small-tipped quartz electrodes, the seal rate was approximately 80%. Seal resistances varied between 16 gigohms and 125 gigohms with the majority being in excess of 50 gigohms. The seals were quite stable some lasting more than 2 hours. From the standpoint of sealability and stability of seals, the quartz proved to be as good or better than most glasses we have tried. Seals in excess of 50 gigohms, when made to cells at the bottom of our approximately 3 mm deep saline-filled chamber, usually resulted in noise levels less than .13 pA RMS at 5 kHz measured through a 4-pole Butterworth filter (highest = .149, lowest = .115). With excised patches or with cells that were not attached to the bottom of the dish, it was possible to lift the electrode so that the electrode tip was within 100-200 microns of the surface of the bathing solution, thus substantially reducing the total capacitance. Under these conditions, noise levels as low as .105 pA in a 5 kHz bandwidth were possible. If the electrode was raised further so that the Sylgard coating no longer touched the solution, we observed that the noise rose substantially and frequently the solution would break free from the pipette uncovering the tip and ending the experiment. To circumvent these difficulties, we coated the surface of the bath with #200 Fluid from Dow Corning (Midland, MI). This dimethylpolysiloxane with a viscosity of 20 centipoise could easily produce an oil layer of 1.0 mm or thicker if sufficient bath fluid was withdrawn to render the surface concave. The low viscosity oil simply ran off the surface of the bath if it was convex in shape. This oil was quite non-toxic to our cells. They maintained their resting voltage and channel activity for at least several hours in a bath covered by this oil.

It was possible to see a sharp demarcation line between the saline and the oil against the side of the immersed pipette. Consequently, the electrode could be raised under direct observation until the tip was just a few microns into the saline. Under these

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circumstances, the non-Sylgarded portion of the tip was surrounded by this silicone oil whose resistive properties appeared to be as good as Sylgard itself. The oil layer also reduced bath evaporation and kept the tip from uncovering from changes in surface tension and so it proved possible to record channel currents for up to 2 hours. With the electrode tip just under the oil, a seal resistance greater than 100 gigohms, and with the channels in the patch closed, it was possible to obtain noise levels as low as .083 pA RMS in a 5 kHz bandwidth. Some representative recordings obtained with bandwidths of 7 and 14 kHz (-3 dB, 8-pole Bessel Filter) are shown in fig. 1.

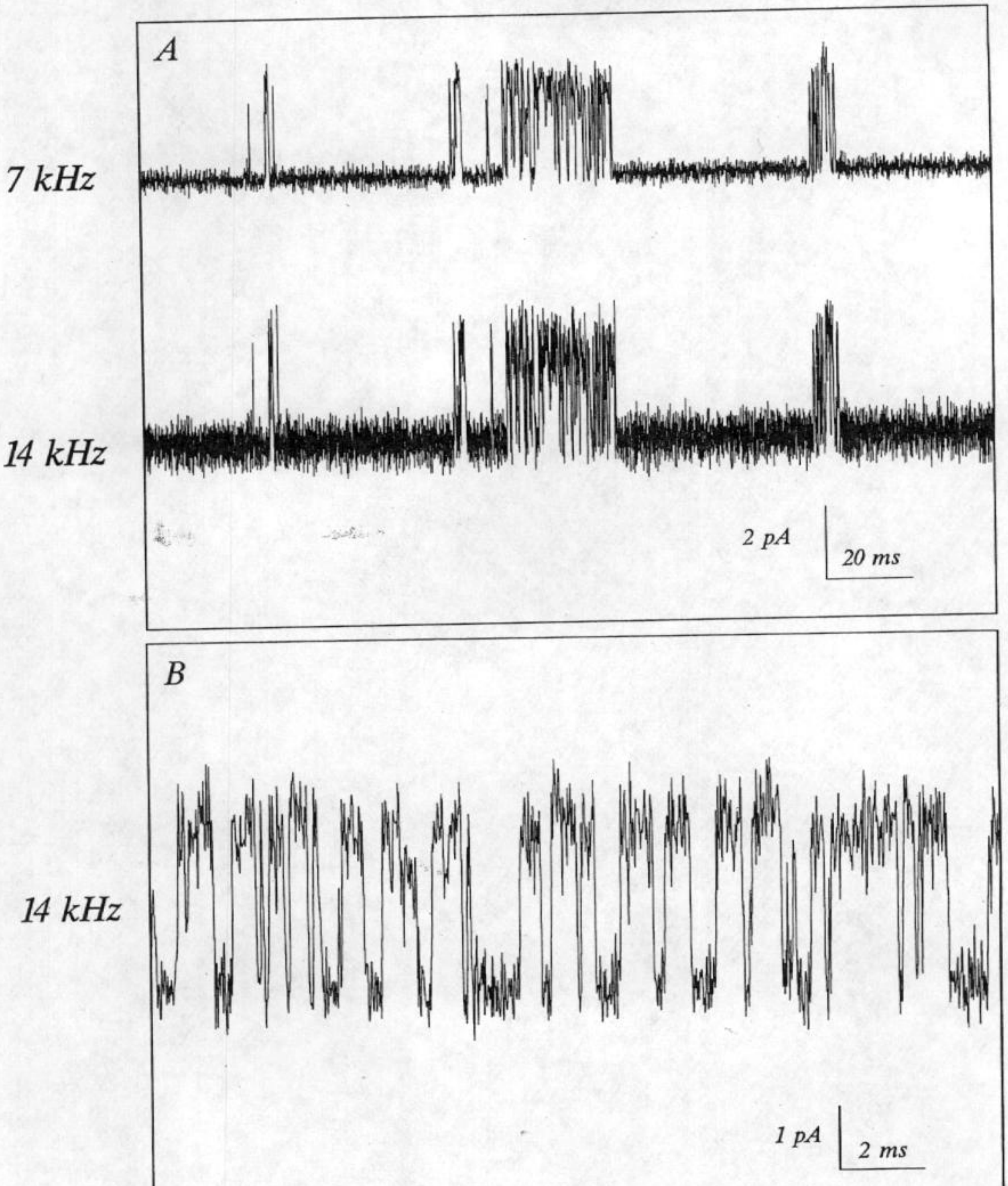
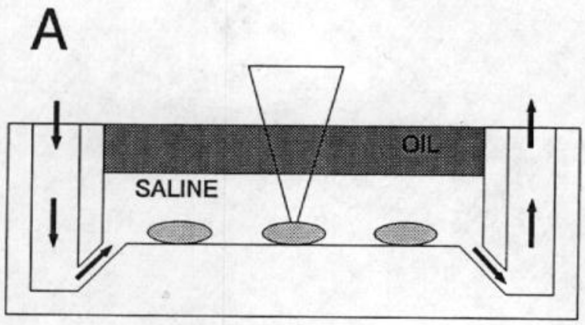


Figure 1: (A) Single channel currents from an excised patch from a rabbit corneal epithelial cell measured using a quartz pipette with its tip within a few µm of a layer of silicone oil (see text for further details of the technique). The pipette contained a 150 mM KCl Ringer and the bath a 150 mM NaCl Ringer. The noise in a 5 kHz bandwidth for this patch was 0.090 pA RMS. The lower trace was recorded at 14 kHz (-3 dB bandwidth), the uppermost bandwidth that would allow essentially error free channel detection using a 50% threshold. The upper trace is the same data following digital filtering (8 pole Bessel characteristics) to yield a final bandwidth of 7 kHz, i.e., half that of the lower trace. As can be seen, the noise has been reduced by slightly more than a factor of two for this two-fold reduction of bandwidth. This is expected from the power spectral density (A^2/Hz) which rises at about $f^{1.4}$ in this range of frequencies. At wider bandwidths the background noise will eventually increase as $B^{3/2}$, where B denotes bandwidth. As the bandwidth is further decreased, the background noise will decrease more gradually, ultimately behaving as $B^{1/2}$ in the range of bandwidths dominated by the white noise associated with the shot noise of the input field effect transistor gate leakage current and the thermal noise of the seal. (B) Single channel currents from the same patch as (A) plotted on an expanded time scale.

When the electrode tip was moved into the oil layer, the noise level fell to equal that when the electrode tip was in the air above the bath. The RMS difference in the noise with the tip in saline just under the oil and with the tip in the oil should be due to seal noise and noise from the pipette resistance in series with the patch capacitance (3). In every case, we found it's magnitude to be very close to the Johnson noise expected from the measured seal resistance.

It was possible to obtain noise levels almost this good in on-cell patches from cells attached to the bottom of the chamber. The chamber that we use (shown in figure 2a) is similar to that described by Daytner, et al., (1). This chamber has 3 wells, one for injecting fluid, one for withdrawing fluid, and the center well for maintaining the cells. When the oil is layered on the central chamber only, it is possible to lower the oil level to near the cell surface by simply withdrawing solution from one of the other two wells. By lowering the oil level to about .2 mm from the cell surface, it was possible to obtain noise levels less than .1 pA RMS in a 5 kHz bandwidth.



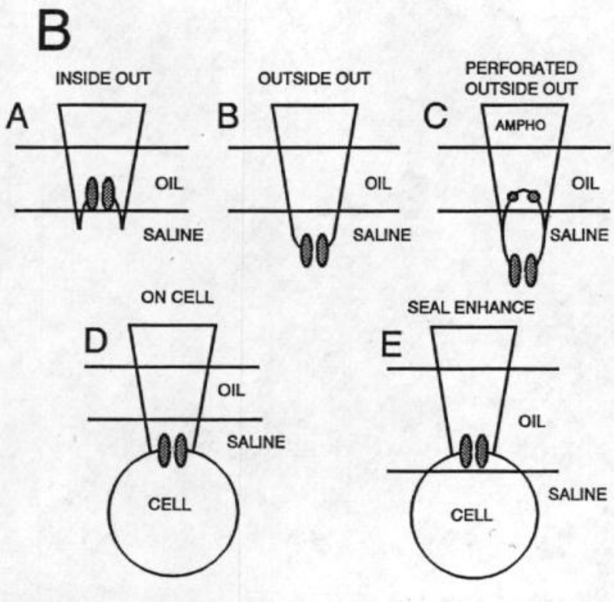


Figure 2: (A) Schematic diagram of the 3-well chamber used. The end wells are connected to the center well at its base through approximately 1 mm slotted ports. (B) Possible patch clamp configurations where the silicone oil layer can be used to reduce noise (see text).

This particular oil had unexpected virtues in that it was possible to pass the electrode through it on the way down to the cells without affecting seal formation. At one time in these experiments, we obtained 10 seals in a row in excess of 100 gigohms from quartz electrodes whose tips had gone through the oil layer previously. With these particular cells, the oil did not seem to have much effect on the channels in the patch. If the electrode tip was drawn into the oil and then placed back into the saline, there was no obvious change in the channel properties due to the oil. This fortuitous finding might not be true for all cells and might not be true for different tip geometries where a larger omega of membrane might allow oil to become trapped in the tip of the electrode.

This combination of using low noise integrating electronics, quartz electrodes, and 200 Fluid silicone oil has resulted in lower background noise levels than heretofore achievable. By using the silicone oil approach, it was possible to reduce the background noise with any of the glasses that we used (Corning 7760, Kimble KG-12) but to date quartz has performed substantially better than these other glasses as expected. Measurements using silicone oil were possible in all the standard patch clamp configurations (fig. 2b). Most provocative perhaps is the possibility that the non-toxic silicone oil might increase seal resistance and thus decrease seal noise if just the electrode tip and the membrane to which it was sealed could be placed in the oil while leaving the remainder of the cell in saline. Because our cells are so small (≈ 6 pF), we have not yet been able to test this configuration but include it for completeness in panel E of fig. 2b. The noise levels we report are low enough that additional improvements will require improvements in the patch clamp electronics and in the electrode holders particularly if techniques are perfected to increase the seal resistance with silicone oil. We expect that the use of the approaches described here will allow most investigators to either reduce their noise at a constant bandwidth or to increase their recording bandwidth by several kHz beyond that which they presently use.

Acknowledgements

Supported by NIH grants EY03282, EY06005, and an unrestricted award from Research to Prevent Blindness.

References

1. Daytner ND, Gintant GA, Cohen IS (1985) Versatile temperature controlled tissue bath for studies of isolated cells using an inverted microscope. Pflügers Arch 403:318-323
2. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85-100
3. Levis RA, Rae JL (1992) Constructing a patch clamp setup. In: Iverson L, Rudy B. (eds) Methods in Enzymology. Academic Press, San Diego, CA, vol. 207, pp 20-66
4. Rae JL, Dewey J, Rae JS, Nesler M, Cooper K (1990) Single potassium channels in corneal epithelium. Invest Ophthalmol Vis Sci 31:1799-1809
5. Rae JL, Levis RA (1992) Glass technology for patch clamp electrodes. In: Iverson L, Rudy B. (eds) Methods in Enzymology. Academic Press, San Diego, CA, vol. 207, pp 66-92