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## Beveling of Fine Micropipette Electrodes by a Rapid Precision Method


*Abstract.* *A technique has been developed for embedding alumina particles 0.05 micrometer in size in the surface of a polyurethane film laid on glass. This abrasive surface is used for rapid, precise, and reliable beveling of Pyrex micropipettes with tip diameters at least as small as 0.1 micrometer. In the snapping turtle retina the beveled electrodes give much better cell penetration and intracellular response stability than unbeveled electrodes of considerably higher electrical resistance.*


The micropipette electrode for intracellular recording has provided a versatile basis for great advances in neurophysiology. Detailed studies, however, have been confined mainly to selected large types of cells, because of the difficulty associated with penetrating small cells without significant damage. The beveling of micropipettes for improved penetration has been reported for tip diameters in the range from 1 to 3 $\mu$m (*1*), and Kripke and Ogden (*2*) have recently described a method for beveling tips to final diameters at least as small as 0.3 $\mu$m. We report here a rapid technique for the precision beveling of still smaller Pyrex micropipettes. These beveled electrodes have shown great advantages for intracellular recording in the snapping turtle retina, and similar advantages may be expected in many other neural preparations.

Kripke and Ogden (*2*) dispersed 0.05-$\mu$m particles of alumina ($Al_2O_3$) in saline on a glass grinding surface that was rotated with negligible wobble, the electrode being lowered by a micromanipulator while the electrode resistance was monitored continuously. Their technique offers many advantages over earlier methods. We found the slow grinding by the freely floating abrasive, however, to be a major limitation. Kripke and Ogden reported that even the rather short and stiff electrode tip formed by a two-stage puller required about 10 to 15 minutes of grinding. With their technique we found that the longer tip formed by the Livingstone puller, which is used by many workers for producing ultrafine electrode tips, required about an hour. These times are a serious inconvenience because a group of electrodes must be prepared for each experiment, preferably shortly before use to avoid damage to the tip by the contained electrolyte. We thus developed a method for embedding the alumina to form an abrasive surface.

We chose polyurethanes as embedding media because they are unaffected by water or saline, resist mechanical wear, and adhere tightly to glass. The two we have used are Varathane (90-gloss) and Humicure in a 50-50 mixture with its solvent (*3*). Both cure to an extremely smooth surface. Varathane is easy to handle and cures adequately for use within 2 days. Humicure requires thorough mixing with its solvent and must be kept moisture-free until use because its curing is catalyzed by moisture from the air, but it cures overnight to an even harder material than Varathane.

A film of embedding medium was first laid upon the flat surface of a glass beam splitter (Edmund Scientific Company, stock No. 578). These beam splitters are 5 mm thick and hence quite rigid, and their size (67 by 83 mm) is convenient for our purposes. The embedding medium may be laid upon either the coated or the uncoated side, the mirrorlike coated side offering some advantage for visualizing the electrode while one is making contact with the abrasive surface. The surfaces to be coated were thoroughly cleaned, eight to ten drops of embedding medium were placed upon the upturned surface of one beam splitter, and a second beam splitter was placed immediately upon it. The embedding medium was thus squeezed into a thin film between the two surfaces. We then slid the upper beam splitter off with the exertion of a slight upward tension. A thin film of embedding medium was thus laid upon both beam splitters, which were set aside in a dustfree cabinet for initial curing.

The alumina was embedded when the polyurethane was viscous enough to be minimally distorted by contact with the alumina, and to flow minimally into the alumina by capillary action, while still sticky enough to hold the alumina. The optimal initial curing time for Humicure varies with humidity but was about 1 hour in our experience, whereas Varathane required somewhat longer. During embedding the partially cured polyurethane film was faced upward, and a pile of alumina was placed on a clean glass surface held slightly above and to one side of the embedding medium. We used Linde alumina B, with a nominal particle size of 0.05 $\mu$m and a specified hardness of 8 on the Mohs scale, Pyrex glass having a lesser hardness of about 5 on that same scale. Gentle air puffs from an empty squirt bottle produced a cloud of powdered alumina, some of which settled upon the polyurethane film, which was then replaced in the dustfree cabinet. When fully cured, it was washed and rubbed by hand to remove all unattached alumina and was ready for use.

Such finely powdered alumina tends to clump, probably because the high surface-to-volume ratio makes electrostatic attractions significant. This was noted by Kripke and Ogden (*2*), and we have also found no way to eliminate this problem entirely. Instead, the clumps of alumina are floated onto the embedding medium only by their own weight. With such light contact and such high viscosity of the polyurethane at the time of embedding, only certain particles of a clump will become embedded, unattached particles being readily removable after the polyurethane has been fully cured. The

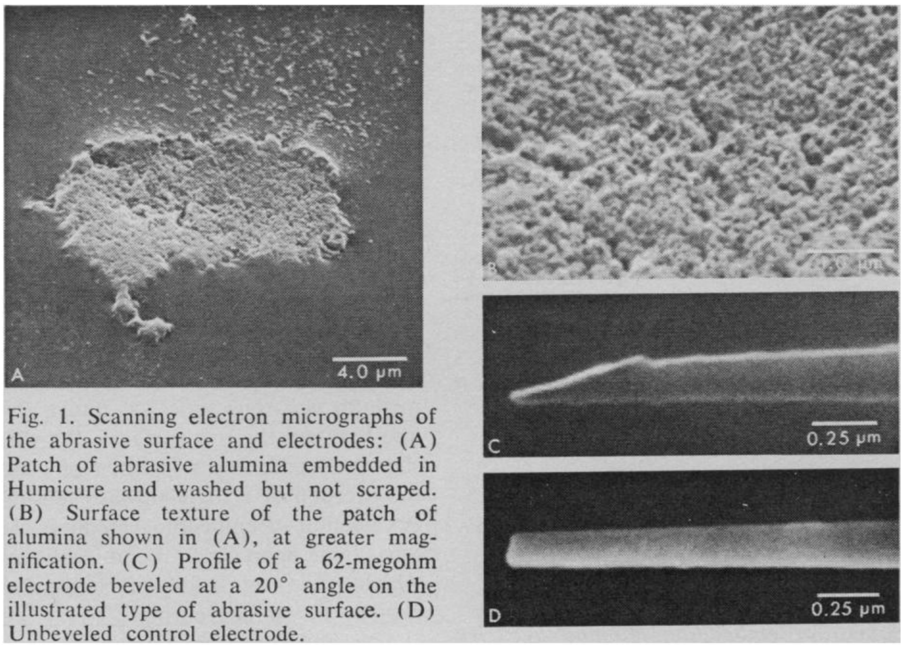


Fig. 1. Scanning electron micrographs of the abrasive surface and electrodes: (A) Patch of abrasive alumina embedded in Humicure and washed but not scraped. (B) Surface texture of the patch of alumina shown in (A), at greater magnification. (C) Profile of a 62-megohm electrode beveled at a 20° angle on the illustrated type of abrasive surface. (D) Unbeveled control electrode.

grinding surface thus formed is very durable, a single one serving for many hundreds of electrodes. If it becomes unusable for any reason, the embedding medium may be softened with toluene and stripped off so that the beam splitter can be reused.

We have used scanning electron microscopy (SEM) to evaluate our abrasive surfaces. Control observations of our embedding media, without abrasive, reveal that their surfaces are smooth and featureless. Even at magnifications as high as about × 25,000, they are indistinguishable from high-quality glass surfaces and hence are ideally smooth for our purposes. Each clump of alumina leaves a patch of embedded particles. These patches vary in size and shape but are otherwise similar; Fig. 1, A and B, shows a typical patch of abrasive at two different magnifications. The abrasive is raised only slightly above the otherwise very smooth Humicure surface, and high magnification shows the texture within the patch of abrasive to be very fine. The slight raising of the abrasive patches above the embedding surface is not a serious problem. Either the electrode tip contacts only the surfaces of abrasive patches, or it rides up over the patches without damage. If desired, a glass scraper, such as the end of a microscope slide with the corners rounded off, may be held at a low angle and used to dress off the higher portions of the abrasive patches.

Our grinding equipment is similar to that of Kripke and Ogden (*2*) but with certain modifications to be described elsewhere (*4*). It provides for rotation of the abrasive surface in the horizontal plane at variable speed (usually 30 to 60 rev/min). The vertical wobble of this surface is less than 1 μm at its outer margin, which is about 6 cm in diameter. The diameter of the circle where grinding occurs is about 2 cm. The electrode may be advanced against the grinding surface at a variable angle (usually 20° to 30°). It is ground in a 0.9 percent (by weight) NaCl solution, with continuous monitoring of its electrical resistance at 130 hertz, which is negligibly different

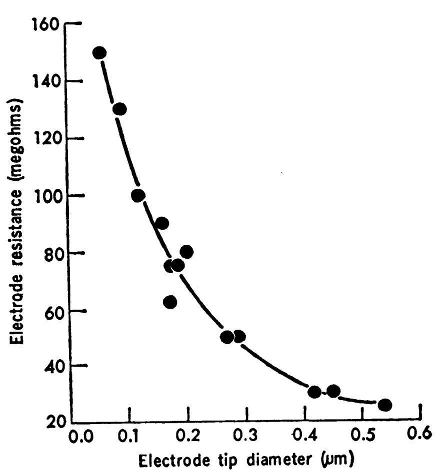


Fig. 2. Electrode resistance as a function of tip diameter. Just after the tip had been beveled, the electrode resistance at 130 hertz was recorded in megohms. Scanning electron microscope photographs were then made, from which the outside diameter of the electrode tip was measured at the base of the bevel, where the cross-sectional area of the electrode's conducting pore is minimal. The curve was drawn by eye through the obtained points.

from the d-c resistance. Most electrodes we have ground have been pulled by a two-stage puller, but the longer tips formed by a Livingstone puller also seem easily handled (*5*). In either case the actual grinding time is very short, seldom exceeding about 15 seconds.

All of the electrodes examined by SEM and used for recording were made from Pyrex glass (Corning No. 7740), the capillary tubing having respective outside and inside diameters of about 1.0 and 0.5 mm; these electrodes were formed by the two-stage puller, filled with 5*M* $KC_2H_3O_2$ by the glass fiber method (*6*), and then beveled on a washed but unscraped abrasive surface. An entire group of such electrodes may be prepared just before an experiment, since 10 to 12 electrodes may be beveled within ½ hour. Our initial electrode resistances have ranged from about 50 to 180 megohms, and beveling reduced the electrode resistance by 10 to 20 percent. The beveling procedure proved reliable as indicated by both SEM observations and the performance of electrodes in intracellular recording. The typical appearance of electrodes by SEM, both with and without beveling, is shown in Fig. 1, C and D. Our methods of mounting and cleaning electrode tips for SEM work permitted high resolution at magnifications up to × 60,000. The beveled electrode of Fig. 1C has an outside diameter of 0.17 μm, measured at the base of the bevel. The cutting edge of the beveled tip is extremely sharp but is distinctly rounded at a diameter of slightly less than 0.03 μm (300 Å). The gold coating (100 to 150 Å) that was required for our SEM observations would make a perfectly sharp tip appear rounded at a diameter twice the thickness of the gold coating. Hence the observed rounding of the tip in Fig. 1C results largely or entirely from the gold coating, and the electrode itself must be even sharper than shown. By contrast, control electrodes consistently had square or almost square ends, an example of the latter being shown in Fig. 1D.

The relation between electrode resistance and tip diameter at the base of the bevel is shown in Fig. 2 for measured tip diameters of 0.06 to 0.54 μm. For electrodes of the type we are using, this permits the tip diameter of any given electrode to be inferred rather accurately from electrode resistance, which is readily measured. The form of this function is of interest because many applications require that either tip diameter or electrode resistance be minimized. Figure 2 shows for our electrodes that, when either tip diameter or electrode resistance has been reduced to a given low value, further decreases can be obtained only at the expense of a very marked and undesirable increase in the other variable.

Ogden (7) finds his beveled electrodes to be more fragile than unbeveled controls when tested in monkey retinas; his beveled electrodes likewise break more readily than controls when cleaned by sonication, as revealed by a reduction of the electrode resistance after breaking. By contrast, we have found no sign of special fragility of the beveled tip, many retinal penetrations being possible in the snapping turtle with no decrease in the penetrating ability of the electrode. Also, during sonication our beveled electrodes proved no more fragile than control electrodes of similar tip size, when electrical resistance was used to match tip size and detect tip breakage. These results suggest that the tip becomes fragile during slow grinding, probably because much flexing weakens the glass. The avoidance of such fragility seems a major advantage of our rapid grinding method.

In the first trial of our beveled electrodes in a snapping turtle eyecup preparation, approaching from the vitreous humor by a Kopf stepping microdrive, we made a single retinal penetration with each of three electrodes with resistances ranging from 80 to 100 megohms (about 0.1 to 0.2 $\mu$m in tip diameter). During each electrode track we obtained intracellular recordings in sequence from a ganglion cell, then from either one or two horizontal cells of the inner nuclear layer, and finally from a photoreceptor. At the end of the third electrode track the photoreceptor recorded from was a cone, as determined by its very small receptive field, its spectral response curve, and the time course of its response to light. In this cell both the resting membrane potential and the light response were entirely stable for 4½ hours. By comparison with conventional electrodes of considerably higher resistance in the same preparation, this consistency of cell penetration and the stability of the intracellular cone response represent dramatic improvements. We infer that the greater response stability results from the beveled electrode cutting a precise aperture through the membrane that is then sealed by the electrode tip. These advantages of beveled electrodes have been fully confirmed in other experiments.

In many small cells of great interest, intracellular recording by conventional electrodes has remained a somewhat marginal technique. The inner segments of snapping turtle photoreceptors, for example, are about 8 to 12 $\mu$m in diameter. In these cells intracellular recording, as applied to photoreceptors to date, is now so reliable that we have even used the technique successfully for a live teaching demonstration. We thus anticipate that for such cells beveled electrodes will bring within technical reach a variety of crucial experiments that have not been possible with conventional electrodes.

At one extreme beveling may be used to increase the diameter of an electrode tip as much as possible while still penetrating a given cell without significant damage. By thus reducing the electrode's electrical resistance, the signal-to-noise ratio is improved for recording small signals. The injection of marker dyes, such as Procion yellow, is also facilitated. Conventional electrodes must have a minimum resistance of about 200 megohms to penetrate well in snapping turtle photoreceptors, but we have already obtained good results from beveled electrodes with resistances as low as 50 megohms. This suggests that beveled double-barreled electrodes for voltage clamping studies should also penetrate readily in this penetration, providing that the beveling axis is appropriately controlled. Relatively large beveled electrodes are also promising for cells covered by connective tissue, since the stiffer but very sharp tip should assist penetration to the cell of interest.

At the other extreme, beveling may be applied to the smallest electrode tips possible to maximize the ease and subtlety of cell penetration. Our experience suggests that this approach would make intracellular recording possible for smaller cells, or smaller parts of cells, than hitherto possible. The range of potential applications in this class is especially great. In retinal work the selection of species is ideally made by criteria other than cell size, partly because the details of retinal circuitry vary greatly between species. The mammalian retina is of special interest, as is the entire mammalian central nervous system, but most cells of these tissues have been too small for intracellular recording by conventional electrodes. Beveling now offers a promising approach to this problem.

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## Eolian Biogenic Detritus in Deep Sea Sediments: A Possible Index of Equatorial Ice Age Aridity

Abstract. *Opal phytoliths and freshwater diatoms, transported mainly in dust to the equatorial Atlantic, are common in sediments deposited when ocean waters were cool, and sparse in those deposited when waters were warm, during the last 1.8 million years. Climate in source areas of the southern Sahara apparently was more arid during glacials and more humid during interglacials.*

Dust transported from Africa by the trade winds to the equatorial Atlantic has been studied for more than 100 years (*1–3*). However, until recently (*4, 5*) little work has focused on the abundance of biogenous and terrigenous components of the dust in equatorial deep sea cores, where variations document changes with time in source areas that can be compared with oceanic temperature fluctuations. To investigate the potential of such comparisons we