showed no specific immunoreactivity. This difference between dorsal root ganglia and spinal cord cultures is consistent with in vivo studies showing that enkephalin is not present in sensory afferent neurons (*15*), but that it is localized to neuronal cell bodies, processes, and presumptive terminals in the dorsal horn of the spinal cord (*5, 6*).

In frozen sections of rat brain, satisfactory visualization of enkephalin immunofluorescence within most of the perikarya that apparently contain enkephalin has required prior treatment of the animals with colchicine (*16*), although several cell body groups may be seen dimly without such manipulations (*6, 17*). In contrast, we have found many examples of immunoreactivity in perikarya of untreated cultured neurons. This demonstration of enkephalin in spinal cord cells grown in tissue culture suggests that these neurons either retain or indeed develop the ability to synthesize opioid peptides while maintained in vitro.

The present results, together with the electrophysiologic responsiveness to enkephalin (*10*) indicate the presence of functional receptors and suggest that dissociated neurons grown in tissue culture may be a suitable model system in which both the chronic application of drugs and physiological manipulations may be effectively used to investigate cellular mechanisms underlying the neuronal functions of opioid peptides.

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The Jet Stream Microbeveler: An Inexpensive Way to Bevel Ultrafine Glass Micropipettes

Abstract. *Ultrafine glass micropipettes can be easily beveled in a jet stream of grinding compound suspended in saline. The beveling is gradual and continuous, highly reliable, and can be accomplished with common laboratory apparatus. The beveled electrodes are comparable in performance to those prepared with expensive commercial bevelers.*

The beveling of glass microelectrodes by grinding has recently become standard practice in many laboratories where intracellular studies are in progress. Beveling increases the electrode pore size and yet permits the retention of small tip dimensions. Such electrodes penetrate tissue more smoothly, have a lowered impedance, and are less noisy than unbeveled electrodes of comparable tip size (*1-3*). The increase in tip pore size that results from beveling is associated with improved ejection of substances by electrophoresis or pressure (*4*), and yet the small, sharp tip facilitates cell penetration (*2*).

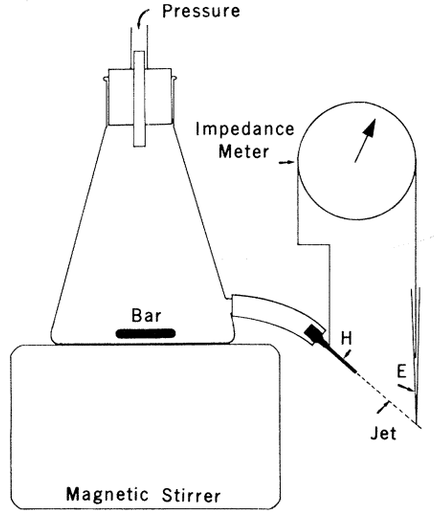

Fig. 1. Diagram of the beveler. A container of micropolish suspension is placed on a magnetic stirrer. Pressure is applied to form a jet of solution from a 20-gauge hypodermic needle (*H*). The impedance meter is connected from the needle to the electrode (*E*), which is held in the jet stream by a micromanipulator.

The commercial electrode bevelers currently available can be traced to the work of Barrett and Graubard (*5*) and the modification of their technique by Kripke and Ogden (*3*) and by Brown and Flaming (*6*). A wobble-free surface in which the grinding compound is embedded is rotated with great precision against an electrode held in a precision advancer. This technique, which requires a substantial investment in equipment and a skilled operator, is inconvenient, results in electrode breakage, and is not suitable for electrodes with long flexible shanks of the type used for most intracellular work.

We have discovered, to our surprise, that very fine beveled electrodes can be produced quickly and reliably if a jet of a grinding solution is simply squirted against the electrode tip. Electrodes thus produced are comparable in performance to those beveled by a rotating grinding surface and we have used them to penetrate and inject horseradish peroxidase (HRP) (E.C. 1.11.1.7) into cells of the frog retina.

The technique we use is illustrated in Fig. 1. As in the earlier procedures, it is essential to monitor electrode impedance during grinding. In essence, the grinding compound (*7*) is directed against the electrode tip at an angle of about 45° in a stream of saline. The suspension is squirted from a 20-gauge hypodermic needle under enough pressure to form a smooth stream. The electrode is lowered into the stream by a micromanipulator of mundane design. Contact with the

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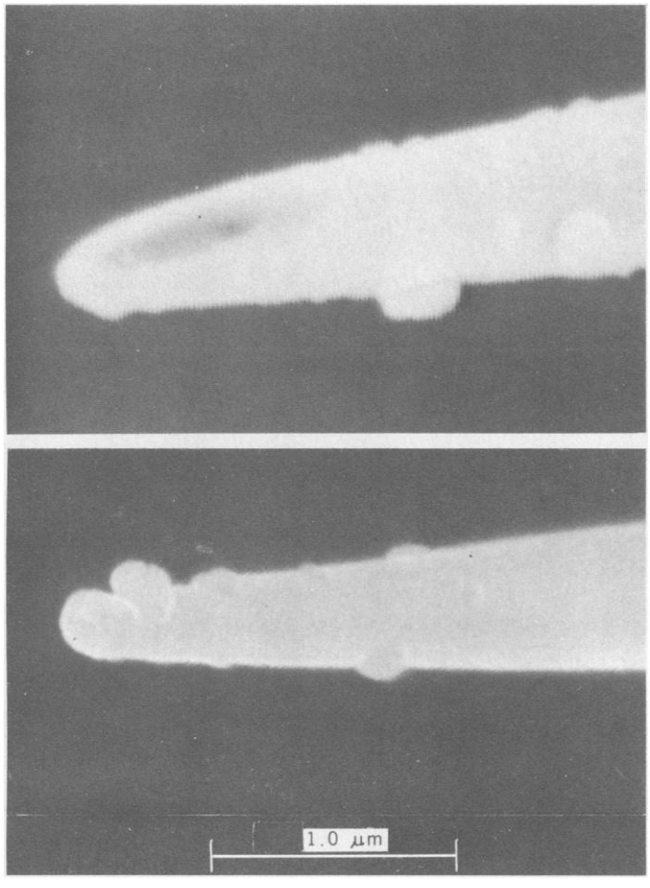

Fig. 2. (Top) Scanning electron micrograph of a micropipette, filled with 0.2*M* KCl, which was beveled from 300 to 100 megohms. (Bottom) Scanning electron micrograph of an unbeveled electrode with an impedance of about 300 megohms. Electrodes are gold-coated.

stream is signaled by an impedance meter. Beveling commences promptly when the pure saline stream is replaced with saline plus grinding compound. Our electrodes, filled with 4 percent HRP and 0.3*M* KCl, usually measure 250 to 400 megohms at first contact and bevel gradually to 100 to 150 megohms in about 5 minutes. No special care is required except that the tip be in the stream; this is indicated by a steady impedance reading.

The saline reservoir containing micropolish is placed on a magnetic stirrer and connected to a 20-gauge hypodermic needle with a short length of tubing at least 0.95 cm in diameter. Since the micropolish settles rapidly, the saline jet contains little or no abrasive when the magnetic stirrer is turned off. Thus the electrode is positioned to obtain a stable impedance reading without agitation. Beveling will start when the stirrer is turned on, at which time the abrasive can be seen to discolor the stream, and is signaled by a gradual reduction in electrode impedance.

Our particular requirement is for an electrode fine enough to permit stable intracellular recordings from frog ganglion cells yet large enough to permit rapid ejection of HRP. Such an electrode is shown in the scanning electron micrograph in Fig. 2 (top). The tip size is about 0.1 μm, despite an impedance reduction of nearly 75 percent. These electrodes are very flexible and are difficult to grind satisfactorily with a grinding surface, and yet they bevel remarkably well in the jet stream beveler. Moreover, they are as robust as unbeveled electrodes [Fig. 2 (bottom)].

Intracellular HRP is an extremely powerful anatomical tool since small injections result in dense light and electron-opaque staining of the entire cell, including even the finest cell processes. Success requires a freely open electrode pore, but even momentary air drying will result in diminished ejection facility (*8*). Thus, we caution those who wish to use this technique to minimize exposure of their electrode tip to air after the grinding has been completed.

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