Controlled bending of high-resistance glass microelectrodes

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HUDSPETH, A. J., AND D. P. COREY. *Controlled bending of high-resistance glass microelectrodes*. Am. J. Physiol. 234(1): C56-C57, 1978 or Am. J. Physiol.: Cell Physiol. 3(1): C56-C57, 1978. —The short working distance of compound microscope lenses in many cases prevents microelectrode penetrations of cells perpendicular to the cell surface. Bending electrodes very near their tips removes this constraint. A method is described for bending glass microelectrodes with a hot filament while their tips are immersed in a water drop. Immersion protects the fine electrode tips from the heat and provides control over the angle through which the electrodes are bent.

compound microscope; intracellular recording; microforge

THE HIGH RESOLUTION of compound microscopes has led to their increased use in electrophysiology, especially for accurate visual control of intracellular microelectrodes. Nomarski differential interference contrast microscopy, in particular, has permitted electrode placement within micrometers of subcellular specializations (1, 3). However, the short working distance of compound microscope objectives (1.6 mm with a 40× water-immersion lens) necessitates introducing electrodes at no more than 30° from horizontal; as penetrations normal to cell surfaces are usually desired, this severely limits work with extended flat preparations such as epithelia or tissue culture cells. Although lenses with working distances of up to 6 mm are available (e.g., the Leitz UMK 50), these have smaller numerical apertures (NA) (0.40) and thus cannot achieve the resolution of short working-distance objectives (NA 0.75). One solution to the problem is to bend the microelectrodes by roughly 90° very close to their tips, so that vertical penetrations are possible with horizontally positioned electrodes. This geometry also permits longitudinal advancement of the microelectrode tip, the most effective for cell impalements, by vertical displacement, the most finely controllable axis on many manipulators.

We have developed a simple, rapid technique for bending high-resistance glass microelectrodes in a controlled manner while protecting their very fine tips. Our apparatus consists of a glass microscope slide with a strip of black plastic tape on its top surface (Fig. 1). A small piece of a slide glued vertically to this surface serves as one boundary for a drop of distilled water; to maintain the curvature of the drop, it is prevented from wetting the tape surface either by a slight grease coating or by a dike constructed from silicone cement. The electrode is held near its base on a lump of modeling clay and its fine end is positioned just inside the drop. When illuminated through the vertical slide and viewed from above with a dissection microscope, the electrode appears bright against a dark background.

Electrodes are bent with a white-hot filament; we use a 0.12-mm platinum wire heated by passing about 3 A of current through it. A foot switch on the current supply leaves both hands free for fine manipulations of the filament and the electrode. As the hot filament approaches the point where the electrode is to be bent,

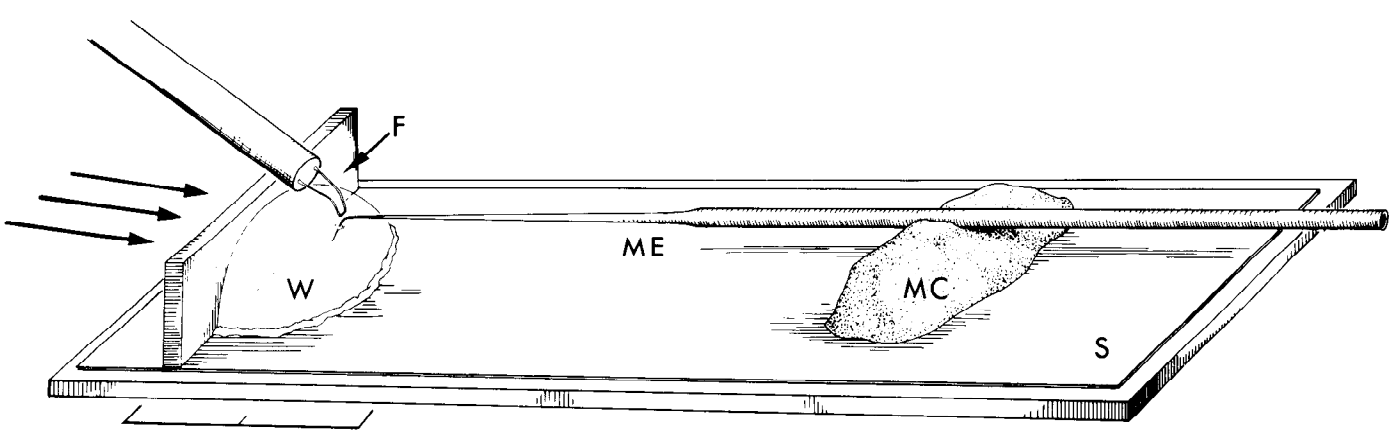

FIG. 1. Drawing of apparatus with electrode shown after bending. Microelectrode (ME) is positioned on slide (S) with a lump of modeling clay (MC). Electrode's tip is inserted in water drop (W) and illuminated from left (arrows). Hot filament (F) is brought close to electrode at desired bending point.

the glass softens focally and the terminal region is pulled perpendicular to the water interface by surface tension. Thus, the position and angle of the bend depend on the placement of the filament and the angle at which the electrode initially enters the drop. One can change the angle of the bend by repositioning the electrode and reheating, or construct more complicated shapes with sequential bends. This method can produce bends as close as 0.2 mm from electrode tips.

The rigidity of the bent electrodes usually suffices for impaling cells, despite the fact that the force of penetration is directed normal to, rather than along, the length of the electrode. When factors such as vigorous perfusion of the experimental chamber necessitate increased rigidity, an electrode may be stiffened before filling by cementing a small (0.2–0.5 mm in diameter) glass fiber along its shank with sealing wax.

Bent electrodes fill as readily as unbent ones, either by boiling or by backfilling with the glass-fiber technique (4). The electrode tips, protected by immersion, are not adversely affected by the bending process. Electrode resistances show no significant changes: test electrodes filled with and tested in 3 M KCl had an average DC resistance of 119 ± 29 MΩ (n = 10) after bending, compared with 132 ± 26 MΩ (n = 10) for unbent controls ($P > 0.2$ by two-tailed t test). Microelectrodes bent in this manner have been used successfully to impale such delicate preparations as vertebrate hair cells (2), cultured cardiac muscle cells, and motor axon terminals in *Drosophila* larvae. Similar electrodes bent without immersion had higher resistances and gave more erratic and less stable penetrations, suggesting that their tips were damaged.

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