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A simple glass-coated, fire-polished tungsten electrode with conductance adjustment using hydrofluoridic acid

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A method is described to produce glass-coated tungsten microelectrodes in 4 simple steps: (1) etching of the wire, (2) coating with glass, (3) fire-polishing, and (4) reopening with hydrofluoric acid to adjust the conductance to a final value. Continuous conductance control is provided during the reopening process by means of an admittance meter to guarantee an exact final adjustment of the conductance required. The complete process yields electrodes of high reliability within a few minutes and the quality of the electrodes remains largely unaffected by any of the manufacturing parameters involved, so that high-performance electrodes are produced without sophisticated procedures. The electrodes have been tested successfully over several years recording from cells in the striate visual pathway of the cat.

Introduction

Experience has shown that extracellular spike potentials from nerve cells are most easily recorded by means of a metal tip microelectrode rather than a fluid-filled pipette (Guld, 1964). Varnished tungsten electrodes produced according to the early method described by Hubel (1957) are used by many laboratories. Since this type of electrode has several disadvantages with respect to mechanical stability and the long-time electrical properties, several methods of insulating electrodes with glass have been described. Forcing the electrode tip through a drop of molten glass as proposed by Wolbarsht et al. (1960) and Guld (1964) requires a difficult heat adjustment since the wire's heat conductivity increases with its thickness. According to the method described by Levick (1972), the etched metal wire is carefully inserted into a previously pulled pipette until the tip protrudes, but this can result in leakage between metal and glass. The conductance of leaky electrodes, however, is in general too high to allow for single-unit recordings. These and other techniques (Baldvin et al., 1965; Merill and Ainsworth, 1972; Merill, 1974) have in common that at least one manufacturing step requires sophisticated procedures. As a result the quality of the electrodes often strongly depends on the skill of the producer. In addition, usually no control of the electrode conductance is provided until the manufacturing process is completed. In contrast to the tip diameter that tolerates a range of 0.5–3 μm, electrode quality, however, depends more strongly on its conductance (Guld, 1964). High conductances (above 0.12 μS) make single-unit recordings impossible while a very low conductance (below 0.06 μS) amplifies the noise level intolerably. Therefore, our aim was to develop a method which would combine ease of manufactur-

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ing with maximum reliability and conductance control of the electrodes during the manufacturing process. In particular, none of the steps to produce an electrode should require critical adjustment of any of the parameters involved.

Method

Four simple steps are required to produce a glass-coated tungsten electrode: (1) etching, (2) coating, (3) fire-polishing, and (4) reopening.

Etching

Tungsten wires of 0.5 mm diameter are etched electrolytically in a saturated solution of potassium-nitrite (Hubel, 1957). The best form for the tip region consists of a shaft of several millimeters length gradually tapering to approximately 50 μm diameter. Over the following 300–500 μm the diameter decreases more rapidly towards the tip. The tip itself should be etched to 1–3 μm. An example of the desired electrode geometry after etching is given in Fig. 1A.

Coating

Coating is performed with a vertical microelectrode puller of any type. First the etched wire is inserted backwards into a glass capillary (1.2 mm outer diameter, 0.69 mm inner diameter, 'Borosilikat Glas' KBF 112069, ZAK, Munich, F.R.G.) and lowered until the end of the wire protrudes enough to allow for contact with the recording equipment. It is fixed in this position with a drop of glue. To melt the glass, a platinum wire (0.5 mm diameter, 120 mm length, heating current approximately 10 A) is coiled 4 turns (around a shaft) with an inner diameter of 3 mm. The electrode is secured in the puller with the tip pointed upwards and approximately 15 mm above the uppermost coil. The pulling procedure starts with a one-minute pre-heating phase to heat up the whole wire. A weak pulling force is then attached to the bottom part of the capillary and pulling should be performed with constant velocity in downward direction so that the procedure lasts 20–30 s. Although this results in a close and gas-free contact between glass and wire, a bead of glass is usually left protruding at the electrode tip (Fig. 1B). If desired, optic control by means of a microscope may be provided during the pulling procedure, but is not necessary.

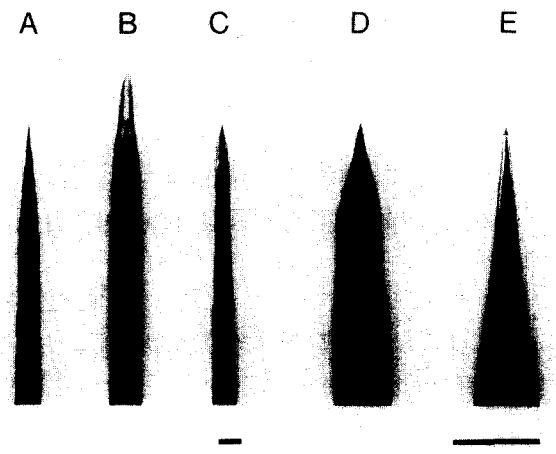

Fig. 1. Examples of electrodes from different manufacturing steps. The bars at the bottom indicate 100 μm. A: etched tungsten wire with a tip of approximately 1 μm. B: glass-coated wire with protruding glass bead. The rough appearance of the wire inside the glass coating is due to refractory effects. C: electrode after fire-polishing has removed glass bead. The tip is still covered by an invisible layer of glass and ready for reopening with hydrofluoric acid. D and E: ready-to-use electrodes shown under higher magnification.

Fire-polishing

The glass bead is removed by plunging the electrode tip into the hottest part of the flame of a Bunsen burner until the tip glows white for just an instant. Due to the high surface tension of the glass, it retracts by fire-polishing in such a way that only a non-visible layer of glass remains coating the remotest part of the tip, thus preventing oxidation (Fig. 1 C–E). Excessive fire-polishing, however, can destroy the tip, so care has to be taken that the tip is heated for only a moment. This guarantees an extremely consistent contact between glass and wire that is necessary for the reopening. Our experience shows that at this stage of the manufacturing process the electrodes may be stored for at least several weeks without loss in recording quality.

Reopening

Up to this time the electrode has been completely covered by glass. To reopen it under conductance control, a microelectrode admittance meter (V. Corti, Zürich, Switzerland; measuring signal: 35 mV rms, 1 kHz sine-wave) is used* and a platinum reference electrode in parallel is immersed in a solution of 40% hydrofluoric acid and a Ringer solution. The sensitive electrode of the admittance meter is attached to the microelectrode to measure the conductance. Several millimeters ($\approx$ 5 mm) of the tip-region of the microelectrode are first immersed into the hydrofluoric acid until the conductance increases. Then the hydrofluoric acid is at once washed away from the tip by dipping it into the Ringer solution and the conductance is remeasured. Note that although the conductance, as measured in hydrofluoric acid, gives an indication of the reopening process, the relevant conductance value has to be measured in Ringer solution. This process should be repeated until the conductance reaches approximately 0.07–0.1 μS equivalent to a resistance of 15–10 MΩ. Finally, the electrodes are washed with distilled water and are now ready to use.

Unevenly coated electrodes would probably not tolerate the mechanical strain during penetration and develop electrical shunts above the tip. During the reopening procedure, however, a large tip region is exposed to hydrofluoric acid and therefore faulty electrodes are sorted out, because they display a sudden increase in conductance indicating a conductive pathway away from the tip.

General remarks

By starting out with a pre-etched wire the complete procedure only lasts about 10 min, thus allowing for its manufacture during the course of an experiment. After use, the electrode wire may be removed for re-use by breaking the glass with a pair of pliers, but care has to be taken that the wire is not bent or split.

* Of course the microelectrode admittance meter can be replaced by a sine-wave generator and an oscilloscope, both of which should be available in every electrophysiological laboratory.

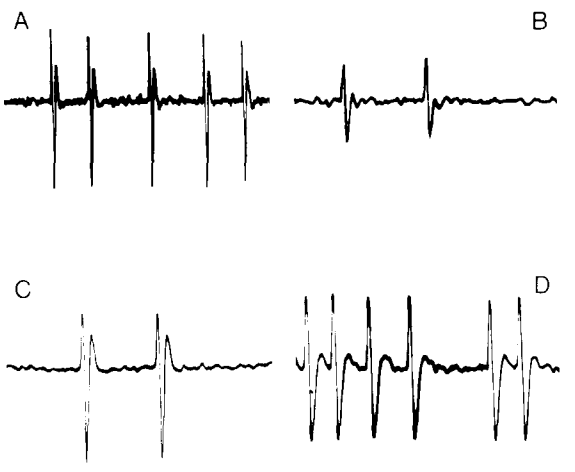

Fig. 2. Typical recordings from the visual pathway of the cat. Time scale bar = 2 ms. A: layer IV S-cells from area 17 of the visual cortex. B: axon recorded in the optic radiation. C: Y-type neuron from layer A of the lateral geniculate body. D: axon of the optic tract. The signal-to-noise ratio is sufficient to allow for single unit recordings and was often better than shown.

Performance test

Starting with the second step (coating), at least 80% of the electrodes pass the complete manufacturing process displaying only minor differences in recording quality. For the last 2 years we have tested the electrodes by recording from cells and axons in the visual pathway of the cat. The bulk of cells was recorded in the visual cortex and displayed spike amplitudes of above 0.5 mV with a background noise level of 0.02–0.04 mV (filtering: low-pass 3 kHz, high-pass 1 kHz). More than 25% of the cells we recorded showed spike amplitudes above 1.0 mV, while amplitudes of more than 5.0 mV were observed occasionally; the highest stable value, 12.0 mV, being reached for a layer IV S-cell. Fig. 2 shows typical recordings from different structures of the visual pathway, demonstrating the general utility of the electrodes.

In the densely packed layer IV of the visual cortex the selectivity of the electrodes was estimated. There, as determined by the different response characteristics of the cells (Eysel et al., 1988), we were able to record from different cells every 30 μm on average. Testing for long-time stability showed that the mean signal-to-noise ratio

after 24 h (several penetrations) had only dropped to approximately 85% of its initial value. This indication of excellent long-term properties was further reflected by remeasuring of the electrode conductance after the experiment. Usually conductance increased only by about 20%, perhaps due to mechanically induced distortions of the glass coating.

Discussion

Over the past several years, diverse procedures to produce microelectrodes have been described (Hubel, 1957; Wolbarsht et al., 1960; Guld, 1964; Marg, 1964; Baldvin et al., 1965; Levick, 1972; Merrill and Ainsworth, 1972; Ainsworth et al., 1977; Braga et al., 1977). In view of that diversity, one may wonder if it is necessary to describe yet another method. However, the earlier methods were usually very elaborate and made high demands on the skill of the producer. By contrast, the ease and reliability of our method was markedly demonstrated when students in our laboratory who had never before seen a microelectrode were able to make high quality electrodes after few hours of training. In particular, a wide margin for any of the parameters involved, such as heat, pulling force or duration of fire-polishing, will not affect recording quality. Thus, we observed that the shape of the glass coat at the tip is not critical for the electrical properties of the electrodes. Another advantage of the method described here is the conductance control during the reopening process. The smooth reopening with hydrofluoric acid allows fine adjustment of the required electrode conductance. Electrical instability of the electrodes arising in conjunction with the use of hydrofluoric acid (Eckhorn, personal communication) was not observed. Such instability, if caused by micro-pores in the glass coating, would probably have been prevented by the extremely homogeneous contact between glass and wire achieved after fire-polishing.

Two years of experimental testing demonstrated the advantages of our manufacturing process and the reliability of the electrodes and showed that the quality of recordings obtained with our electrodes is comparable to that obtained with fiber electrodes (Reitböck, 1983).

In conclusion, we believe that the described procedure represents an improvement in terms of reliability and simplicity and has the advantage that it can be performed with standard equipment available in every electrophysiological laboratory.

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