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A simplified method for manufacturing glass-insulated metal microelectrodes

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Abstract

A simplified method to manufacture durable, glass-insulated, tungsten microelectrodes with sufficient control of the final electrode impedance is described. This method requires only two instruments, an electrolytic etcher for wires and pipette puller, for manufacturing these electrodes. The manufacture of these electrodes involves 3 steps: (1) etching tungsten wire to sharpen the tip, (2) insulating the electrode by pulling a glass pipette over the sharpened tungsten wire and (3) assessing and adjusting the tip exposure and impedance of the electrode to meet recording requirements. Control over the electrode impedance is easily accomplished by varying the distance between the uppermost portion of the heating coil and the sharpened wire tip before a glass pipette is pulled over the wire tip. This distance determines the area of tip exposure and also the location where the glass insulation ends and the exposed electrode tip begins. A performance test of these electrodes in a chronically prepared monkey showed that they were strong enough to repeatedly penetrate thickened dura mater without significant changes in impedance and to isolate cortical neuronal activity after these multiple penetrations. Furthermore, the strength of these microelectrodes eliminated the need to remove reactive granular tissue from the dura overlying the recording site.

Key words: Microelectrode; Tungsten wire; Glass insulation; Chronic animal preparation; Extracellular single-unit recording

1. Introduction

A variety of methods is used routinely to manufacture microelectrodes for extracellular recording of neuronal activity. Microelectrodes have been formed from stainless-steel (Blum and Johnston, 1969), elgiloy (Quintana and Fuster, 1986), tungsten (Hubel, 1957; Marg, 1964; Baldwin et al., 1965; Freeman, 1969; Levick, 1972; Loeb et al., 1977; Bullock et al., 1988; Wörgötter and Eysel, 1988), iridium (Salcman and Bak, 1976: Loeb et al., 1977) and platinum-iridium (Wolbarsht et al., 1960; Guld, 1964; Kinnard and Maclean, 1967; Merrill and Ainsworth, 1972) wires. Insulating materials used to cover the electrode have included varnish (Hubel, 1957; Marg, 1964; Bartlett, 1966; Kinnard and Maclean, 1967; Blum and Johnston, 1969), epoxy (Freeman, 1969; Ciancone and Rebec, 1989), vinyl (Spinelli et al., 1970), parylene (Salcman and Bak, 1976; Loeb et al., 1977) and glass (Wolbarsht et al., 1960; Guld, 1964; Baldwin et al., 1965; Levick, 1972; Merrill and Ainsworth, 1972; Ainsworth et al., 1977; Quintana and Fuster, 1986; Bullock et al., 1988; Wörgötter and Eysel, 1988). When compared to electrolyte-filled glass micropipette electrodes, metal electrodes offer several advantages for extracellular recording. Electrodes with metal tips are stronger than glass micropipettes and can record extracellular unit activity for longer time periods and with larger amplitude (Wolbarsht et al., 1960; Guld, 1964). Currently, the most commonly used metal microelectrode is an epoxy-coated, tungsten wire that has been sharpened by electrolytic etching.

For extracellular recordings using awake, chronically prepared animals, the durability of the electrical insulation covering the metal electrode is especially important because the dura mater overlying the recording site thickens over time (Lemon, 1984). This can abrade and strip the insulation off the metal surface during

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electrode penetration. Therefore, an electrode that can readily penetrate this thickened dura mater to reach the target site with minimal disturbance of its insulation and electrical recording properties is highly desirable. Loss of insulating material causes a reduction in electrode impedance and in the amplitude of recorded action potentials. In the worst case, the damaged electrode is unable to isolate unit activity at the target site in the brain or spinal cord. Unfortunately, loss of insulation is often a problem with epoxy-coated tungsten microelectrodes.

In contrast to epoxy, glass makes an excellent insulating material for metal electrodes by providing a smooth (low friction) and durable (high density) surface (Wolbarsht et al., 1960; Baldwin et al., 1965; Merrill and Ainsworth, 1972; Ainsworth et al., 1977; Wörgötter and Eysel, 1988). The main disadvantage of glass-coated, metal microelectrodes is the difficulty in their manufacture (Spinelli et al., 1970; Merrill and Ainsworth, 1972; Bullock et al., 1988; Wörgötter and Eysel, 1988). The most common methods used for insulating metal electrodes with glass involve a 2-step process: (1) applying the glass coating onto the sharpened wire, and (2) removing the glass from the sharpened end of the wire to expose the electrode tip. The classical methods for glass insulation were described by Wolbarsht et al. (1960) and Guld (1964). These methods involve placement of the electrode tip through a bead of heated solder glass (high lead) which requires the difficult task of maintaining an optimal glass temperature and which often results in imperfect insulation of the electrode (Levick, 1972; Quintana and Fuster, 1986; Wörgötter and Eysel, 1988). Levick (1972) proposed inserting the tip of an sharpened metal wire into a sharpened glass micropipette. However, this often introduces an additional problem, namely, formation of a gap and fluid leakage between metal and glass at the electrode tip (Wörgötter and Eysel, 1988). All of these above procedures require considerable skill and training time to make consistent high-quality microelectrodes in sufficient quantity. However, Baldwin et al. (1965) and Merrill and Ainsworth (1972) reported an easier process for glass-coating metal electrodes by heating and pulling a glass capillary tube over the sharpened wire tip. Nevertheless, re-exposing the electrode tip with strong acid as performed by Wörgötter and Eysel (1988) can be a difficult task for the inexperienced investigator.

The present paper describes a surprisingly simple adaptation of the methods described by Merrill and Ainsworth (1972) to manufacture high-quality, glass-insulated, tungsten microelectrodes in large numbers. Also, the method allows the operator to adjust easily the exposed electrode tip area and thus to determine the electrode impedance. Unlike previous methods, the additional step to re-expose the electrode tip by removing glass insulation is unnecessary. Moreover, only two instruments that are commonly available in most electrophysiology laboratories are required in the manufacturing process: an electrolytic etcher for wires and a micropipette puller. We will also present evidence of the electrode's durability when used in multiple penetrations of thickened dura mater and cerebral cortex and of the electrode's capability of recording cortical cell activity after such penetrations.

2. Methods

2.1. Electrode manufacturing procedure

The construction of glass-insulated tungsten microelectrodes is a 3-step process: (1) electrolytic etching of tungsten wire to form a sharpened tip, (2) insulating the electrode by pulling a glass pipette over the sharpened tungsten wire and (3) assessing and adjusting the tip exposure and impedance of the electrode to meet recording requirements.

2.1.1. Tungsten wire etching

Straight tungsten wire (0.25 mm diameter) is cut into 50 mm lengths. Each of 4 wires in an array is held with a clamp attached to the electrode etcher (BAK Electronics, Model EE-ID). At the beginning of the etching process, the wire is submerged in a 2 M sodium hydroxide solution such that 10 mm and 27 mm lengths of wire, respectively, are submersed at the maximum upward and downward strokes of the dipping cycle. From our experience, the best tip shapes are obtained by using DC currents with a voltage of 3.4–3.7 V (9–10 V setting on the electrode etcher) passed through the wires and solution. Ten submersion and withdrawal cycles (dips) are used to dissolve at least 10 mm of the original length of the wire. Using these settings, the wire will start from a distance of 16 mm to taper gradually toward its sharpened tip and the tip will be etched to less than 1 μm diameter. Sharpened tungsten wires are washed in an ultrasonic cleaner (Bronson, Bronsonic B-12) with double distilled water for 15 min and then washed in the sonicator with 100% ethanol for 15 min.

2.1.2. Glass coating etched tungsten wire

The sharpened tungsten wire is inserted into a borosilicate glass capillary tube (A-M Systems, No. 6255; 0.4 mm inner diameter, 0.75 mm outer diameter, 150 mm length) from the blunt unetched end until this end protrudes 3–5 mm from the glass capillary tube. This allows a short uninsulated wire end for connection to electrophysiological recording equipment. The protruding blunt wire end is then fixed to the glass capillary with a drop of glue (DURO super glue, cyanoacry-

late ester). A vertical pipette puller (David Kopf, Model 700C) is used to cover the sharpened wire end with glass insulation. To melt the glass, a coiled titanium wire is the most suitable heating element (David Kopf, No. 710T) since it does not deform with repeated use. However, titanium coils are not readily available because they are no longer manufactured. We currently use a nichrome wire heating element that is 1 mm in diameter and coiled 2.5 turns into a cylindrical shape with a diameter of 5 mm (David Kopf, No. 710). However, the nichrome coil requires minor reshaping after many heating cycles. As shown in Fig. 1A, the glass capillary pipette encasing the tungsten wire is held in the puller clamps with the sharpened wire tip pointing upward. Special attention must be given to adjusting the distance between the sharpened wire tip and the uppermost portion of the heating coil (distance D in Fig. 1A). This adjustment determines the exposed tip area and electrode impedance (see below). It is also important to adjust the upper and lower clamps so that the glass-encased tungsten wire is held precisely parallel and centered in relation to the vertical axis of the heating coil. This prevents the sharpened wire tip from touching the capillary glass wall and from possible bending when pulled vertically along with the glass. The current used for heating the nichrome wire is 25 A (75 setting on puller heating control dial) and the solenoid height is adjusted to the 'standard' position of this pipette puller (3.9 mm from its extreme uppermost position). Since there is no current monitor in the solenoid circuit, trial and error adjustment is the only method to determine the most appropriate parameter. We set the solenoid control dial at 35 on this puller model. When all parameters of the pipette puller are correctly set, the glass should melt and collapse onto the sharpened end of the wire after pulling the glass pipette apart. The opposing tip of the separated glass pipette should remain within the core of the heating

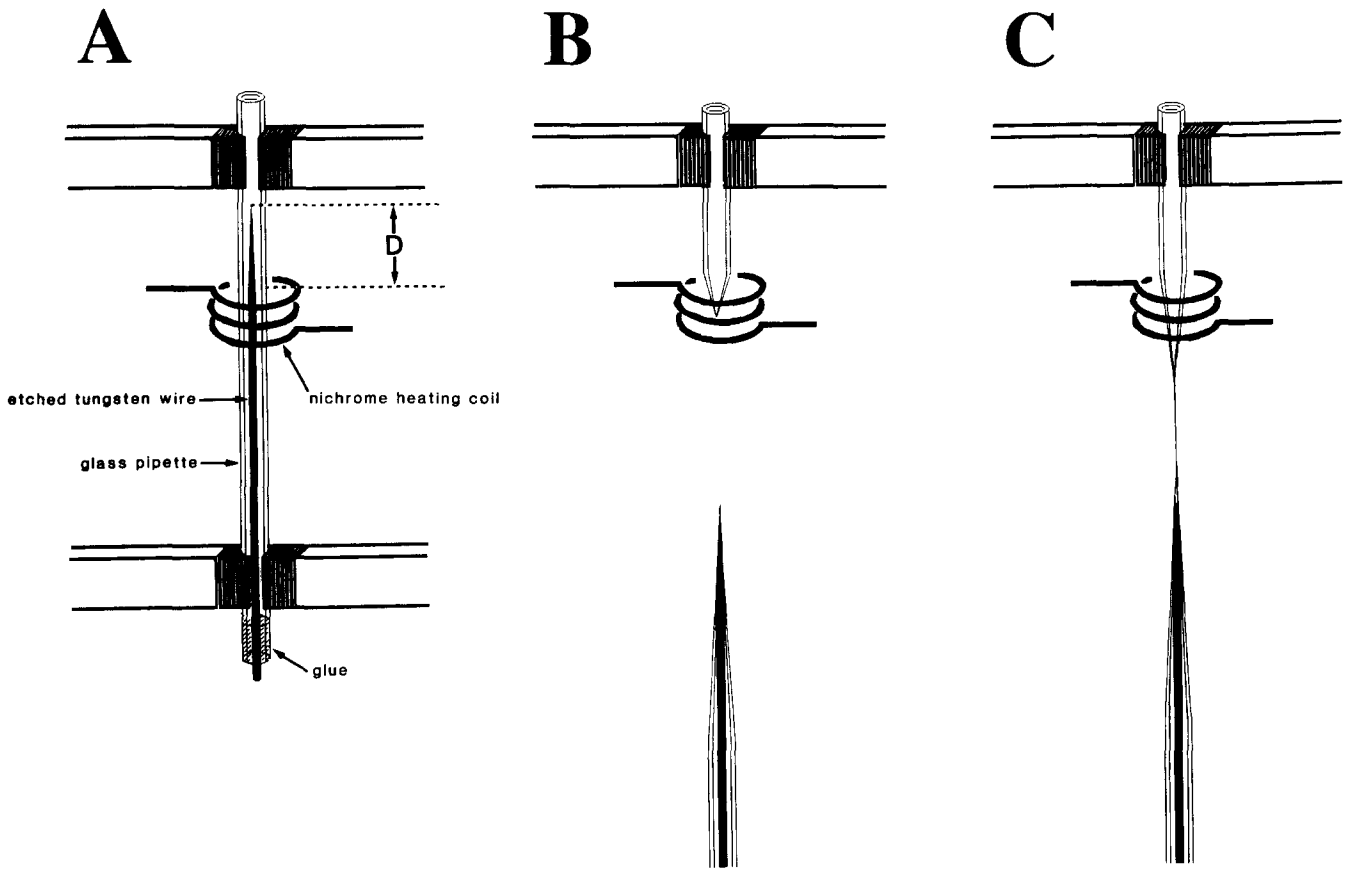

Fig. 1. Glass insulation of sharpened tungsten wire. A: relative pre-pull positions of sharpened wire, glass pipette and heater coil. The sharpened wire was fixed inside a borosilicate glass capillary with a drop of glue so that the blunt end protruded 3–5 mm from the glass capillary. The glass capillary encasing the tungsten wire was held by the vertical pipette puller clamps with the sharpened wire tip pointing upward. The distance (D) between the uppermost portion of the heating coil and the sharpened wire tip determined the length of tip exposure and thus the electrode impedance. B: relative post-pull positions of materials and apparatus (successful insulation). When all electrical parameters of the pipette puller and relative position of materials and apparatus were correct, the glass melted and collapsed onto the tungsten wire after the pipette pull. The tip of the separated glass pipette that did not contain the sharpened tungsten wire remained within the core of the heating coil. C: relative post-pull positions of materials and apparatus (unsuccessful insulation). If the solenoid pulled too slowly or the coil heating current was too high, the glass pipette did not break and a continuous string of glass joined the separated pipette segments.

coil (see Fig. 1B). If the solenoid pulls too fast, or the coil heating current is too low, the glass pipette will break too early and a large portion of the sharpened wire tip will be exposed. If the solenoid pulls too slowly or the coil heating current is too high, the glass pipette will not break and a continuous string of glass will join the separated pipette segments (see Fig. 1C).

2.1.3. Checking electrode shape and impedance adjustment

The impedance of each electrode is checked using an impedance meter that utilizes a 1 kHz sine wave in its test current (BAK Electronics, Model IMP-1). The shape of the electrode tip is examined under a light microscope with a magnification of 400×. If the electrode is pulled successfully, the glass insulation terminates about 15–30 μm from the sharpened wire tip. The uninsulated portion of the electrode can be readily distinguished from the insulated portion of the electrode by its darker appearance when appropriately illuminated under a light microscope (Fig. 4).

Electrode impedance is determined by the length of the exposed electrode tip. The relationship between the length of exposed electrode tip and its impedance is shown in Fig. 2. The best fit of the function was a logarithmic decay curve ($y = 6.46 - 3.39 \times \log(x)$; $r^2 = 0.949$). The electrode impedance can be adjusted by changing the distance between the sharpened wire tip and the top of the heating coil (i.e., distance D in Fig. 1A) before a glass pipette is pulled over the wire tip. For example, if the impedance of an electrode is higher than desired, the impedance of the next electrode can be readily decreased by raising the sharpened wire tip in relation to the top of the coil before pulling the pipette. This effectively increases the length of the exposed electrode tip. Conversely, impedance is increased by lowering the sharpened wire tip in relation to the top of the coil before the pipette pull and consequentially, decreasing the length of the exposed electrode tip. The consistency of our method for glass coating the etched tungsten wire was demonstrated by comparing the exposed tip lengths and impedances in our most recent batch of electrodes. In 45 consecutively manufactured electrodes, 43 had glass insulation that terminated 15–30 μm (average: 23.87 ± 3.98 μm SD) behind the tungsten wire tip and had an average impedance of 1.61 ± 0.37 MΩ (SD). The remaining two electrodes had exposed tip lengths of 32.5 and 37.5 μm.

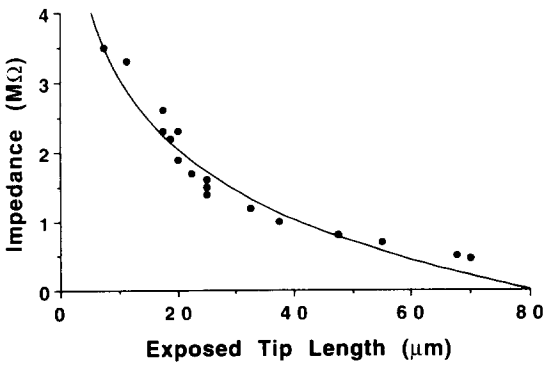

Fig. 2. Relationship between exposed tip length and electrode impedance. Impedance was measured using an impedance meter that utilized a 1 kHz sine wave in its test current. The mathematical function that best fits these data was that of a logarithmic decay curve.

2.2. Performance test

Ten electrodes with closely match impedances (1.52 ± 0.11 MΩ; mean ± SD) were used for the performance test. These electrodes were used to penetrate the dura mater and cerebral cortex of a monkey that was implanted 9 months earlier with a microelectrode positioning device. A stainless steel, internally threaded ring mount (2 cm diameter) for attaching a *X-Y* slide positioner and hydraulic piston microdrive was implanted on the skull overlying a craniotomy hole and the posterior parietal cortex. An externally threaded ring with an attached disk of silastic sheeting (1 mm thick) was inserted into the ring mount to provide a water-tight seal. The entire assembly of rings was capped with a nylon plug; the inner ring with the silastic disk attachment was replaced each week. During the 9 months following exposure of the cerebral cortex, the formation of reactive granular tissue overlying the exposed dura was covered by weekly reapplication of a thin layer of antibacterial ophthalmic ointment. No attempt was made to remove this tissue.

Performance of the electrodes was evaluated while the monkey was anesthetized with 0.7% halothane balanced with oxygen. The monkey was pretreated with atropine sulfate (0.04 mg/kg, s.c.) and a single dose of ketamine (10 mg/kg, i.m.) was administered to permit tracheal intubation and to facilitate anesthetic induction with halothane. Rectal temperature and end tidal CO_2 concentration was monitored and maintained within normal limits. Each electrode was attached to the microelectrode positioning device. Four penetrations through the meninges and cerebral cortex were made for each electrode; the penetrations were equally spaced in the anterior-posterior and medial-lateral directions at 0.5 mm intervals. The impedance of each electrode was measured by an impedance meter after each penetration. Significant changes in mean impedances across the 4 penetrations for all 10 electrodes were determined by 1-way analysis of variance (ANOVA). In addition, recordings of cortical activity in the inferior parietal lobule were made for each electrode during the 1st and 4th penetrations in order to assess their ability to isolate and hold cells for study.

These extracellular recordings were AC-amplified, filtered (300 Hz to 3 kHz bandpass), monitored on a digital oscilloscope, stored on video tape and played-back for producing hardcopies.

At the end of the experiment the monkey was overdosed with pentobarbital sodium and then perfused transcardially with normal saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The dura mater with reactive granular tissue overlying the recording site and the intact dura mater from another site were removed and placed in buffered 4% paraformaldehyde for 72 h. The fixed pieces of dura were embedded in paraffin and then cut in cross-section. The sections were stained with trichrome stain and examined using a light microscope.

3. Results

The mean impedances of 10 electrodes before any penetration and after each of 4 penetrations are shown in Fig. 3. The electrode impedance (mean ± SD) after the 0th, 1st, 2nd, 3rd and 4th penetrations were 1.52 ± 0.11, 1.43 ± 0.12, 1.43 ± 0.14, 1.40 ± 0.15 and 1.46 ± 0.16, respectively. An ANOVA revealed no significant differences ($P > 0.05$) across the mean electrode impedances obtained prior to any penetration and after each penetration.

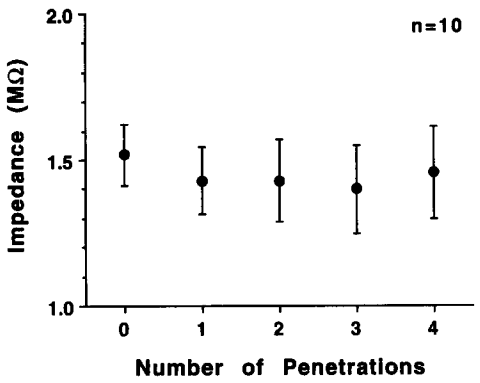

Fig. 3. Impedance of the electrodes before any penetrations and after each of 4 penetrations through the silastic disk, the dura mater and the cerebral cortex of a chronically prepared monkey. Each dot and bar represents the mean and standard deviation of the impedance of 10 electrodes. There were no statistical differences in the mean impedances obtained prior to any penetration and after each penetration (ANOVA, $P > 0.05$).

Shown in Fig. 4 is an example of 1 electrode's tip before any penetration (Fig. 4A, upper) and after 4

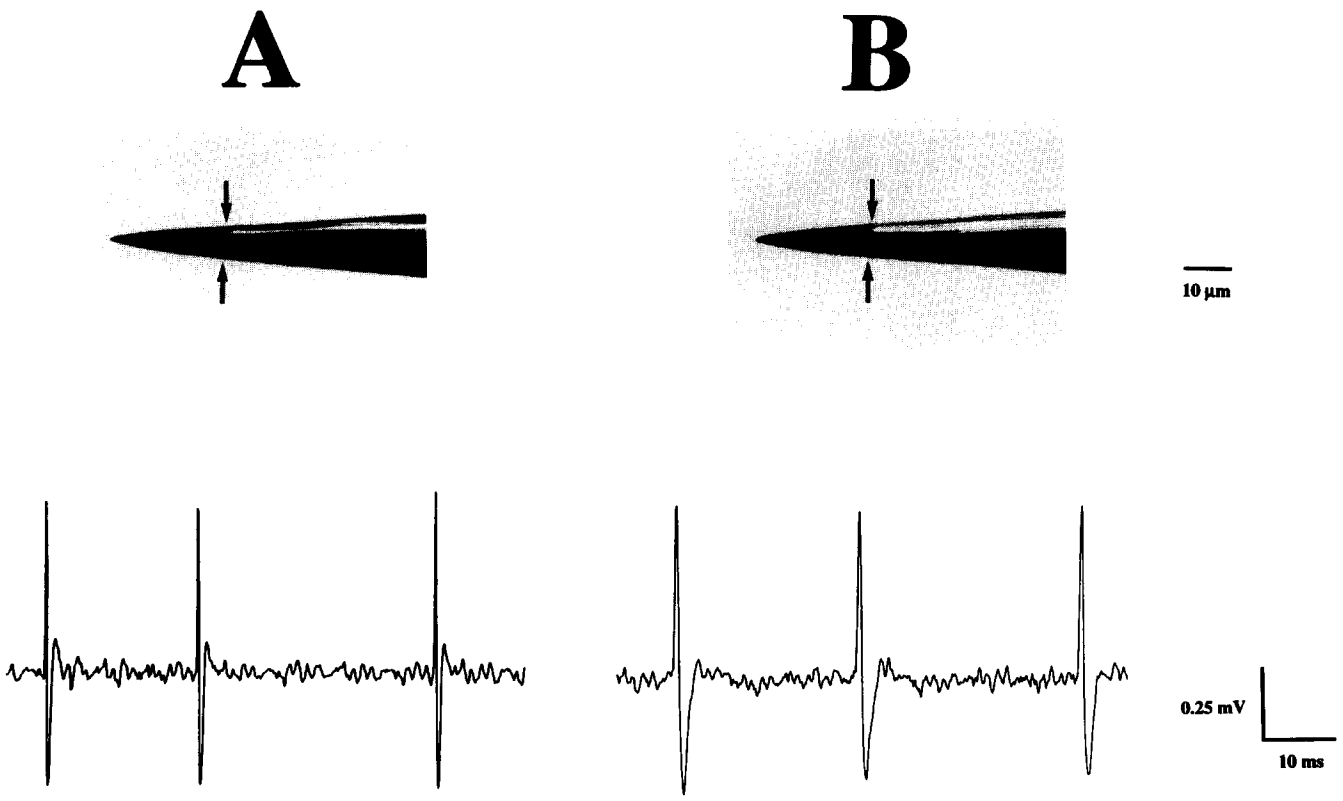

Fig. 4. Example of an electrode tip before any penetration (A, upper) and after 4 penetrations through a silastic disk (1 mm), granular reactive tissue, thickened dura mater and cerebral cortex (B, upper). Activities of two different cortical neurons were recorded in the grey matter of the inferior parietal lobule by the same electrode during the 1st penetration (A, lower) and 4th penetration (B, lower). The junction between the glass insulation and bare electrode tip is indicated with arrows. Note that the glass insulation remained intact after multiple penetrations. The peak-to-peak spike amplitudes of these units isolated during the 1st and 4th penetrations were 1.01 mV and 0.98 mV, respectively. These signals were at least 14 times larger than the background noise (0.07 mV).

penetrations (Fig. 4B, upper) through the meninges and cerebral cortex. Note that the position of the glass insulation and bare tip border (indicated by arrows) was unchanged by multiple penetrations. Activity of two different cortical neurons recorded in grey matter of the posterior parietal lobule during the 1st and 4th penetrations accompany the photographs of the electrode's tip. The amplitudes of unit activity recorded during the 1st (Fig. 4A, lower) and 4th penetrations (Fig. 4B, lower) were 1.01 mV and 0.98 mV, respectively; these were at least 14 times larger than background noise (0.07 mV). It is evident from the photographs of the electrode tip that the glass tightly enveloped the tapered surface of the tungsten wire and terminated 25 μm from its tip without forming any observable edges. The transition between glass and tungsten surfaces (arrows in Fig. 4A and B, upper) was seen best by illuminating the electrode tip with oblique lighting and light microscopy at high magnification (400 $\times$).

Histological examination of the meninges overlying the recording site showed reactive thickening of the dura mater and also epidural, reactive tissue growth (Fig. 5A). This epidural, reactive tissue formation was comprised of two layers. The outer layer was usually covered with antibiotic ointment and a silastic disk but was often exposed briefly to the extracranial milieu. It was composed of many capillaries and some collagen fibers (Fig. 5A,a). Cells such as polynuclear neutrophils and macrophages and many plasma cells which are

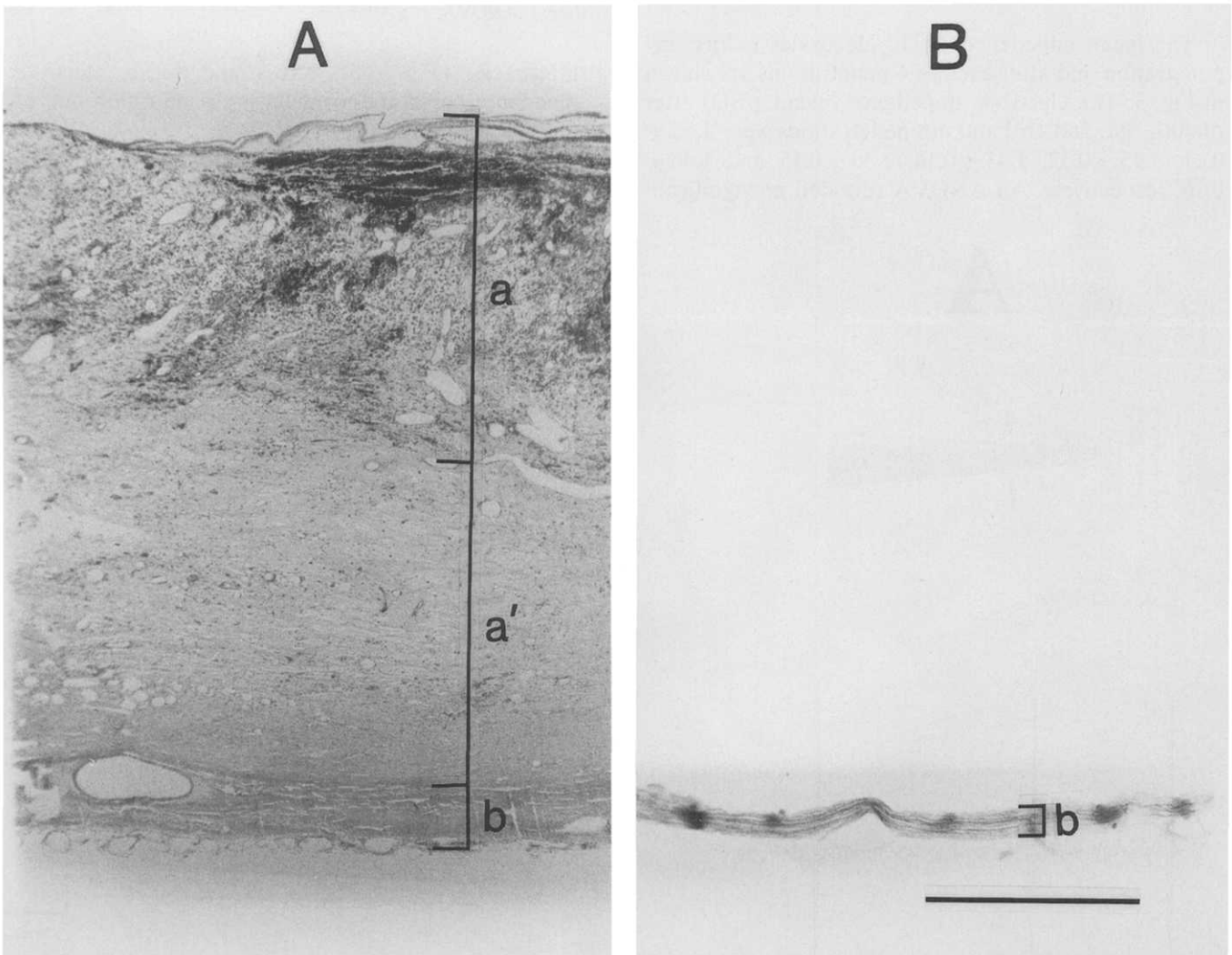

Fig. 5. Histology of the monkey dura mater overlying the cortical recording site (A) and at a distant non-recording site (B). The tissue was cut in cross-section and stained with trichrome. Upper side of each photograph indicates outer portion of the dura; lower side indicates inner portion of the dura. In A, the dura overlying the recording site showed reactive thickening (A,b) in contrast to normal dura (B,b), and development of reactive granular tissue growth (A,a and a'). The reactive granular tissue formed into two main layers. The outer layer (A,a) was comprised of many capillaries and some collagen fibers. Inflammatory cells also accumulated in this layer. The inner layer (A,a') was comprised mainly of collagen fibers. Inflammatory cells were rarely observed within the inner layer. Scale bar: 1 mm.

associated with chronic and acute inflammatory processes were found within this outer layer. On the other hand, the inner layer was composed primarily of collagen fibers (Fig. 5A,a'). Inflammatory cells were rarely seen in this layer which indicated that the inflammatory process was no longer active within this layer. The total thickness of the reactive dura and overlying granular tissues was 3.50 mm and was nearly 20 times thicker than that of normal dura which was 0.18 mm (compare Fig. 5A,B). It should be emphasized that the electrodes used for the performance test were required to penetrate not only the thickened dura and reactive granular tissue but also the overlying 1-mm-thick silastic disk.

4. Discussion

A simple method of manufacturing glass-insulated tungsten microelectrodes that does not require procedures to expose a glass-covered, sharpened wire tip has been described. We have successfully used these electrodes for extracellular recording of neurons in both the cerebral cortex of behaviorally trained, awake monkeys (Dong et al., in press), and the caudate-putamen of anesthetized rats (Chudler et al., 1993).

A method for glass insulation of metal electrodes by collapsing a glass capillary tube onto metal wire was first reported at least 53 years ago (see Baldwin et al., 1965). Merrill and Ainsworth (1972) were the first to use a micropipette puller to manufacture glass-insulated tungsten electrodes. Wörgötter and Eysel (1988) described methods to expose a glass-covered wire tip by removing the glass with fire-polishing and hydrofluoridic acid. Bullock et al. (1988) described a complex workstation for manufacturing glass-coated tungsten microelectrodes which incorporated the methods used by Merrill and Ainsworth (1972) and Ainsworth et al. (1977). In spite of these improvements, the glass-covered wire tip must still be exposed by using heat (Baldwin et al., 1965), mechanical force (Baldwin et al., 1965; Levick, 1972), or DC current (Wolbarsht et al., 1960; Guld, 1964), by dropping the tip into molten glass (Merrill and Ainsworth, 1972; Bullock et al., 1988), by polishing paper (Quintana and Fuster, 1986) or by hydrofluoridic acid (Wörgötter and Eysel, 1988). Exposure of the glass-covered wire tip can be a difficult task that may require extensive training and skill. Moreover, even with adequate tip exposures it still required additional steps to plate platinum or gold onto the electrode tip to reduce the impedance and the electrical noise of the electrode (Levick, 1972; Merrill and Ainsworth, 1972). It appears that our success in exposing the microelectrode tip is attributed to the relationship between the diameter of the glass pipette and tungsten wire and to the position of glass-encased wire tip and puller heating coil prior to pulling the pipette over the wire tip. The inner diameter and wall thickness of the glass pipettes that we used are the smallest reported. Moreover, the pipette inner diameter and wire diameter are more closely matched than previously described (Baldwin et al., 1965; Merrill and Ainsworth, 1972; Wörgötter and Eysel, 1988). Without the need to expose a glass-covered wire tip, the manufacture of glass-insulated tungsten microelectrode is simplified so that little training is needed. Also, less time is required for the glass insulating procedure. By starting with a sharpened and cleaned wire, the entire process of glass coating each wire can be completed within 3 min.

While the levels of peak-to-peak noise in recordings with our microelectrodes (≈ 70 μV) were higher than the noise levels reported in recordings with other microelectrodes (e.g., Merrill and Ainsworth, 1972), the high signal-to-noise ratio permitted us to effectively record from cells in the cerebral cortex of awake monkeys (Dong et al., in press) and anesthetized monkeys. The high signal-to-noise ratio of activity seen in Fig. 4 was commonly obtained with our electrodes.

Our method does not require the purchase of manufacturing equipment such as a microforge, an oven for baking the epoxy or instruments for exposing the electrode tip or plating the tip with platinum chloride or gold chloride. Moreover, it does not require personnel to handle hazardous chemicals such as sodium cyanide for etching platinum wire or hydrofluoridic acid to remove glass. Both of these chemicals must be handled in a fume hood and necessitate the protection of rubber gloves and masks to prevent skin contact and inhalation of vapors.

When epoxy-coated tungsten microelectrodes are used to record from cortical neurons in awake monkeys, the reactive tissue from the dura over the recording site must be removed on a biweekly basis to prevent abrasion and loss of the epoxy coating and to avoid bending the electrode tip and consequently, reduction of electrode impedance. This requires not only time to perform the surgery but it also exposes the animal to several risks such as complications related to anesthesia, mechanical injury to the cerebral cortex, bleeding from hypervascular reactive tissue and the chance of infection. The reactive granular tissue growth is a protective reaction against infection from outside of the body, thus removal of these tissues reduces this protective activity. Our glass-insulated tungsten microelectrodes have eliminated the need to remove reactive tissue from the dura since these electrodes can readily penetrate the dura without significant impedance changes. Moreover, these microelectrodes can also be gas sterilized for recording in chronically prepared animals without any effect on electrode impedance.

Acknowledgements

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