ELSEVIER

Journal of Neuroscience Methods 68 (1996) 247–251

JOURNAL OF NEUROSCIENCE METHODS

An easily constructed carbon fiber recording and microiontophoresis assembly

Jianjun Fu, Joan F. Lorden *

Department of Psychology and Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Received 31 January 1996; revised 3 June 1996; accepted 4 June 1996

Abstract

Traditional multibarreled pipette electrodes are still widely used in microiontophoresis studies. Although better electrodes have been introduced, they are difficult and time-consuming to make. Construction of a carbon fiber recording and microiontophoresis assembly is described here. The construction of a carbon fiber electrode assembly is easy and carbon fibers have excellent characteristics for recording extracellular single unit activity. Signal-to-noise ratio of the carbon fiber electrode is maintained when combined with microiontophoresis. The electrode assembly can be used repeatedly, if cleaned properly, and it can be used to make marking lesions upon completion of an experiment.

Keywords: Microelectrode; Microiontophoresis; Extracellular single-unit recording; Carbon fiber; Electrolytic lesion

1. Introduction

Microiontophoresis is widely used in neuropharmacological studies, especially when combined with extracellular single unit recording in anesthetized animals. Great improvement has been made to microiontophoretic electrodes since the early application of this technique (for reviews see Hicks, 1984; Stone, 1985). The traditional multibarreled microiontophoretic electrode is easily pulled from commercially available multibarreled glass stock. Although quickly and easily constructed, these electrodes suffer from several disadvantages. Among these is noise coupling when ejection currents pass through drug barrels. Iontophoretic artifact makes it difficult to assess the effect of iontophoretically applied substances on neuronal activity (Crossman et al., 1974).

An important modification of the multibarreled electrode is the 'piggy-back' assembly. With this approach, a conventional recording electrode is glued side-by-side to a multibarreled pipette for microiontophoresis. The tips of the recording electrode and the microiontophoretic pipette are separated to reduce capacitative coupling. Although this arrangement improves the signal-to-noise ratio in comparison with the traditional multibarreled microiontophoretic pipettes, fabrication of a piggy-back assembly is a tedious multi-step procedure. Refinements of this approach continue to be published (Li et al., 1990; Godwin, 1994; Verberne et al., 1995).

Carbon fiber electrodes, both single and multibarrelled, were originally introduced by Armstrong-James and Millar (1979). They and others also introduced the use of carbon fiber electrodes in voltammetry (Armstrong-James and Millar, 1979; Kruk et al., 1980; Millar et al., 1981; Gonon et al., 1981; Armstrong-James et al., 1981; Crepsi et al., 1984). Carbon fibers have excellent characteristics for recording extracellular unit activity, and multibarreled carbon fiber electrodes are ideal for single unit recording combined with microiontophoresis. However, the application of this type of electrode in microiontophoresis studies has been limited. In a search of the Medline database for studies using microiontophoretic electrodes, we retrieved 104 studies published in English from 1991 to 1996 in which microiontophoresis was combined with extracellular single unit recording in the brain. While 72 (69%) studies reported the use of the traditional multibarreled microiontophoretic pipettes, 29 (28%) used piggy-back assemblies, and only 3 (3%) used carbon fiber electrodes (Armstrong-James et al., 1991, 1993; Armstrong-James and Callahan, 1991). Thus, despite its disadvantages, the traditional multibarreled pipette is used much more frequently than other types of microiontophoretic electrodes. This suggests

* Corresponding author. The Graduate School, University of Alabama at Birmingham, 701 20th Street South, Birmingham, AL 35294, USA. Tel.: (205)-975-8852; Fax: (205)-975-7189; E-mail: JLorden@gradschool.huc.uab.edu

Published by Elsevier Science B.V.

that investigators may be avoiding the improved, but difficult to construct, electrode assemblies in favor of an easily manufactured electrode. We now describe modifications in the manufacture of multibarreled microiontophoretic carbon fiber electrodes that simplify production while maintaining superior recording properties. We also report the use of carbon fiber electrodes to make marking lesions at the completion of experiments, facilitating verification of electrode placement.

2. Methods

2.1. Materials

Commercially available multibarreled borosilicate glass tubing (World Precision Instruments, Sarasota, FL, USA) was used to make carbon fiber electrodes. We used five-barrel tubing with a filament in each barrel to facilitate filling. The internal diameter of each barrel was 0.68 mm. Carbon fibers with a diameter of 8 μm were obtained from Amoco Performance Products, Itasca, IL, USA. For preparation of the recording electrode, a small bundle of carbon fibers was cut to about the same length as the multibarreled glass tubing. Fine forceps with their tips covered with small pieces of polyethylene tubing were used to pick up a single carbon fiber.

Usually the carbon fiber was inserted into the center barrel of multibarreled glass tubing. 'Wet' and vacuum methods for the insertion of carbon fibers into glass tubing have been described previously (Armstrong-James and Millar, 1979; Anderson and Cushman, 1981). In the more commonly used wet method, the tubing is first filled with acetone, ethanol, or distilled water as a lubricant. The carbon fiber is then pushed through the tubing in short steps. Because of their small diameter and flexibility, the carbon fibers are difficult to insert in this manner. We used a much easier 'dry method'. A metal wire was cut slightly longer than the multibarreled glass tubing and inserted into the center barrel of a multibarreled glass tubing. A single carbon fiber was then isolated and attached to the wire with a conductive glue (Silver Print, GC Electronics, IL, USA). The wire was used to draw the attached carbon fiber into the tubing until it occupied approximately three-fourths of the tubing. A small drop of cyanoacrylate glue was then applied to fix the wire to the wall of the center barrel of the pipette. The wire protruding from the center barrel was used to make a connection with the recording equipment. The diameter of the metal wire can vary as long as it fits the glass tubing and is easy to connect to the recording equipment. We used a Diamel coated nickel-chromium wire with a diameter of 62 μm, with the insulation removed from both ends.

After insertion of the carbon fiber into the center barrel, the multibarreled tubing was pulled in a vertical pipette puller (Kopf Instruments, Tujunga, CA, USA). A four and one-half turn heater coil was made from 8 gauge nichrome wire. The inner diameter of the coil was 5 mm. Once the tubing was pulled, the part of the pipette containing the wire was retained and the remainder was discarded. The carbon fiber was left protruding from the tip of the center barrel.

2.2. Adjusting carbon fiber length

Excess carbon fiber can be removed from the electrode tip by cutting. However, the carbon fiber is brittle and it is difficult to control the tip length by cutting. We have tested two alternative methods to remove excess carbon fibers.

In the first method, a small hook of copper wire (1–2 mm diameter) was glued to a Plexiglas block small enough to put on a microscope stage. A droplet of normal saline was placed on the hook which could viewed under a ×100 magnification microscope objective. The electrode was placed on a microscope slide and advanced into the saline. The outputs of an AC transformer were connected to the copper hook and the wire lead of the carbon fiber electrode. When AC (5–8 V, 60 Hz) was applied, the carbon fiber was gradually 'etched' producing a pointed tip similar in shape to a commercially available parylene coated tungsten electrode (see Fig. 1). The etching speed can be adjusted by changing the voltage applied. On average, it takes about 2 min. Too high a voltage may cause the carbon fiber to shake and crack its glass coating. An intact glass coating is important for maintaining the signal-to-noise ratio during microiontophoresis.

Armstrong-James et al. (1980a,b) described a similar method for etching carbon fiber electrodes, but recommended the use of chromic acid rather than the saline suggested here. The effect of chromic acid on the surface of the carbon fiber tip is unknown. In our experience, however, the use of saline as an etching solution produced electrodes that were less noisy and which had better recording characteristics than those produced using chromic acid. In addition, there is less chance of contaminating the iontophoretic barrels when saline is used.

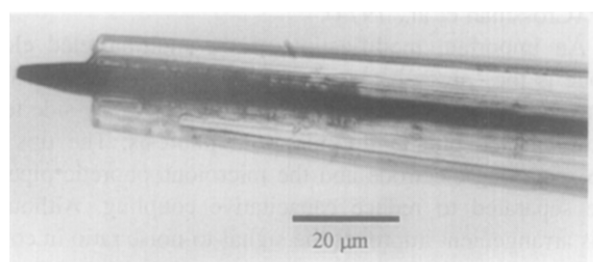

Fig. 1. A multibarreled carbon fiber microiontophoretic assembly. Four drug ejection barrels surround the carbon fiber containing barrel. The pointed tip was produced by etching with AC current in normal saline solution.

We also tested the application of DC current as a method to cut the tip of the carbon fiber. A setup similar to that described above was used, except that the copper wire hook that held the saline droplet was connected to the anodal outputs of a DC constant current lesion maker (Grass Instruments, Quincy, MA, USA, output voltage 150 V). The cathode was connected to the wire lead of the electrode. A 500 μA cathodal DC current was applied to the electrode. As the carbon fiber touched the saline surface, a small spark was observed under the microscope, as the carbon fiber was shortened. The electrode was advanced slowly toward the saline until the desired tip length was achieved. This method provided better control over the carbon fiber tip length than etching. Also, the use of DC current shortened the carbon fiber more rapidly than the use of AC current.

2.3. Breaking an uneven glass tip

When the pipette puller is properly set, it is not difficult to achieve glass tips of a uniform size. If the openings of drug delivery barrels are too small, the electrode can be etched until the carbon fiber end shrinks into the glass tubing. The glass tip can then be broken to a suitable length.

To break the glass tip, the method previously described by Merrill and Ainsworth (1972), Hellier et al. (1990), and Godwin (1994) was used. A platinum wire (diameter 0.5 mm) was bent into a small loop and glued to a block of Plexiglas. Both ends of platinum loop were connected to the outputs of an AC transformer. A bead of glass powder (Corning no. 7570) suspended on the loop was heated to melting with AC current (2–4 V, 60 Hz). Under microscopic control, the electrode was gradually advanced into the melted glass bead until a desirable tip length was reached. The heating current was then turned off. Contraction of the glass bead during cooling fractures the glass tip producing a clean and even break with the carbon fiber exposed. It is important to shorten the carbon fiber so that it is completely within the tubing before breaking the glass. Otherwise, contraction of the glass bead during cooling either breaks both carbon fiber and the glass tip, leaving no carbon fiber exposed, or the glass tip breaks leaving the carbon fiber stuck to the glass bead. In the latter case, trying to separate the carbon fiber from the glass bead usually damages the electrode.

2.4. Making lesions with carbon fiber electrode

We have used the electrode described to record in several brain regions. In order to verify the location of the recording site, we have made lesions by passing DC current through the carbon fiber electrode. A Grass DC constant current lesion maker was used to apply 50 μA (150 V) of cathodal DC current to the electrode tip for 5–10 s.

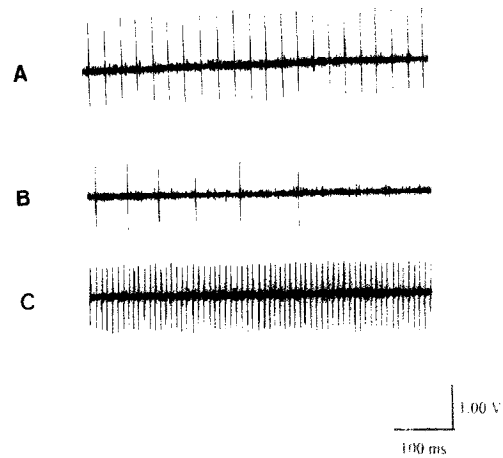

Fig. 2. Extracellular single unit activity recorded from a neuron in rat deep cerebellar nucleus. The recording quality remained unchanged during microiontophoretic application of drugs. Panel A shows spontaneous activity of the cell; B, activity during microiontophoretic application of GABA at 100 nA, showing that the cell activity slowed down and then stopped completely; and C, cell firing rate increase during application of bicuculline at 100 nA.

3. Results and discussion

The carbon fiber electrodes described have been employed in extracellular single unit recording studies combined with microiontophoresis in the deep cerebellar nuclei (DCN) and cerebellar cortex of the rat. Fig. 2 shows a recording from a DCN neuron. Fig. 2A shows the spontaneous activity of the cell, and Fig. 2B, activity during microiontophoretic ejection of GABA at 100 nA. The neuron's activity slowed down and stopped. Fig. 2C shows the increase in activity that occurred during the application of bicuculline at 100 nA. Notice that during microiontophoretic application of drugs, the recording quality was unaltered. Recording quality has been examined over a range of ejection currents from 20 to 100 nA and found to be stable.

Construction of a carbon fiber microiontophoretic electrode is fast, simple and reproducible. With practice, a carbon fiber electrode can be produced in about 10 min. With the methods described above, the exposed carbon fiber tip is easy to trim and the glass tip is easy to break to a specific diameter. In addition, the carbon fiber microiontophoretic electrode offers several practical advantages over piggy-back assemblies. First, the 'V' shape of piggy-back assembly makes it unsuitable for reaching relatively deep neural structures. The small profile of the carbon fiber microiontophoretic electrode causes less damage to surrounding tissue during electrode penetration, making it advantageous for use at subcortical sites. Second, in piggy-back assemblies, the tips of both the drug delivery pipette and the recording electrode are extremely fine and flexible. It is difficult to ensure the proper alignment of the recording electrode and multibarreled tubing (Verberne et al., 1995). Control over the distance between the tips of the electrode and microiontophoresis pipette is poor. This, in

turn, may be an important factor influencing the apparent sensitivity of neurons to iontophoretically applied substances. In comparison, there is relatively little variation over the distance between recording tip and drug release site with the electrode described.

Another advantage of the multibarreled carbon fiber microiontophoretic electrode assembly is that it can be cleaned and reused. A carbon fiber electrode can be used repeatedly until its tip is damaged. To clean a multibarreled carbon fiber electrode, 3–5 V cathodal DC is briefly passed through the electrode while its tip is placed in a saline solution. The current required to clean a multibarreled carbon fiber electrode is slightly greater than that required to clean a metal electrode, 2–3 V DC.

Marking the site of electrode placement is frequently necessary for in vivo neuronal activity recording experiments. The most commonly used marking methods are dye injections through micropipettes and electrolytic lesions made with metal electrodes. With carbon fiber electrodes, a dye-lesion method has been used (Todd and Millar, 1983; Fox and Armstrong-James, 1986). A 2.5–4 μA cathodal DC current was applied for 7–10 s followed by a dye injection from one of the iontophoretic barrels of the electrode. It is not clear whether the mark was a lesion or a dye-stained spot in this case. Sawaguchi et al. (1986) reported that with carbon fiber electrodes, cathodal DC current at 10–20 μA for 10–20 s failed to mark the recording site. Instead, they found that anodal DC current could deposit carbon in the tissue to form a small mark, rather than making an electrolytic lesion. More recently, electrolytic lesions have been made with carbon fibers by increasing cathodal DC current (Armstrong-James et al., 1991). In our experiments, we used relatively large amounts of DC current to mark the recording sites. In a series of experiments, lesions were successfully made in 42 out of 45 rats with cathodal DC current at 50 μA for 5–10 s. These lesions were easily identified under low magnification. The shape of the lesion was usually oval, similar to the electrolytic lesions made with metal microelectrodes. Fig. 3 shows a lesion made with 50 μA of cathodal DC current for 10 s. When the electrode was examined after the lesion, no deterioration was observed at the tip of the carbon fiber electrode under microscopic examination.

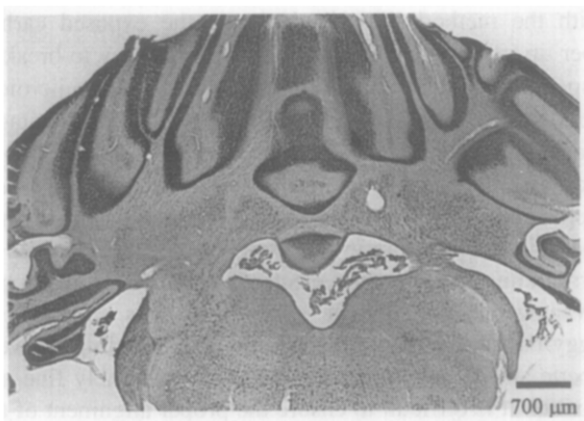

Fig. 3. A unilateral electrolytic lesion made with 50 μA cathodal DC current for 10 s is shown in the right medial nucleus of the cerebellum of a rat.

The ability to make marking lesions, combined with ease of construction, reproducibility, and superior recording characteristics make carbon fiber microiontophoretic assemblies the method of choice for in vivo microiontophoresis studies.

Acknowledgements

This study was supported in part by a grant from the Dystonia Medical Research Foundation. The authors thank Dr. Franklin Amthor for providing samples of carbon fibers and both Drs. Amthor and Dwayne Godwin for useful discussion.

References

Anderson, C.W. and Cushman, M.R. (1981) A simple and rapid method for making carbon fiber microelectrodes, J. Neurosci. Methods, 4: 435–436.

Armstrong-James, M. and Callahan, C.A. (1991) Thalamocortical processing of vibrissal information in the rat. II. Spatiotemporal convergence in the thalamic ventroposterior medical nucleus (Vpm) and its relevance to generation of receptive fields of S1 cortical 'barrel' neurones, J. Comp. Neurol., 303: 211–224.

Armstrong-James, M. and Millar, J. (1979) Carbon fibre microelectrodes, J. Neurosci. Methods, 1: 279–287.

Armstrong-James, M., Callahan, C.A. and Friedman M.A. (1991) Thalamocortical processing of vibrissal information in the rat. I. Intracortical origins of surround but not centre-receptive fields of layer IV neurones in the rat S1 barrel field cortex, J. Comp. Neurol., 303: 193–210.

Armstrong-James, M., Fox, K. and Millar, J. (1980a) A method for etching the tips of carbon fibre microelectrodes, J. Neurosci. Methods, 2: 431–432.

Armstrong-James, M., Fox K., Kruk, Z.L. and Millar, J. (1981) Quantitative iontophoresis of catecholamines using multibarrel carbon fibre microelectrodes, J. Neurosci. Methods, 4: 385–406.

Armstrong-James, M., Millar, J. and Kruk, Z.L. (1980b) Quantification of noradrenaline iontophoresis, Nature, 288: 181–183.

Armstrong-James, M., Welker, E. and Callahan, C.A. (1993) The contribution of NMDA and non-NMDA receptors to fast and slow transmission of sensory information in the rat SI barrel cortex, J. Neurosci. 13: 2149–2160.

Crepsi, F., Paret, J., Keane P.E. and Morre, M. (1984) An improved differential pulse voltammetry technique allows the simultaneous analysis of dopaminergic and serotonergic activities in vivo with a single carbon-fibre electrode, Neurosci. Lett., 52: 159–164.

Crossman, A.R., Walker, R.J. and Woodruff, G.N. (1974) Problems associated with iontophoretic studies in the caudate nucleus and substantia nigra, Neuropharmacology, 13: 547–552.

Fox, K. and Armstrong-James M. (1986) The role of the anterior intralaminar nuclei and *N*-methyl D-aspartate receptors in the generation of spontaneous bursts in rat neocortical neurones, Exp. Brain Res., 63: 505–518.

Godwin, D.W. (1994) A tungsten-in-glass iontophoresis assembly for studying input-output relationships in central neurons, J. Neurosci. Methods, 49: 211–223.

Gonon, F., Buda, M., Cespuglio, R., Jouvet, M. and Pujol, J.F. (1981) Voltammetry in the striatum of chronic freely moving rats: detection of catechols and ascorbic acid, Brain Res., 223: 69–80.

Hellier, M., Boers, P. and Lambert, G.A. (1990) Fabricaton of a metal-coated multi-barrel microiontophoresis assembly, J. Neurosci Methods, 32: 55–61.

Hicks, T.P. (1984) The history and development of microiontophoresis in experimental neurobiology, Prog. Neurobiol., 22: 185–240.

Kruk, Z.L., Armstrong-Jame, M. and Millar, J. (1980) Measurement of the concentration of 5-hydroxytryptamine ejected during iontophoresis using multibarrel carbon fibre microelectrodes, Life Sci. 27: 2093–2098.

Li, B.M., Mei, Z.T. and Kubota, K. (1990) Multibarreleded glass-coated tungsten microelectrode for both neuronal activity recording and iontophoresis in monkeys, Neurosci. Res., 8(3):214–219.

Merrill, E.G. and Ainsworth, A. (1972) Glass-coated platinum-plated metal microelectrodes, Med. Biol. Eng., 10: 662–672.

Millar, J., Armstrong-James, M. and Druk, Z.L. (1981) Polarographic assay of iontophoretically applied dopamine and low-noise unit recording using a multibarrel carbon fibre electrode, Brain Res., 205: 419–424.

Sawaguchi, T., Matsumura, M. and Kubota, K. (1986) Long-lasting marks of extracellularly recorded sites by carbon fiber glass micropipette in the frontal cortex of chronic monkeys, J. Neurosci. Methods, 15: 341–348.

Stone, T.W. (1985) Microiontophoresis and Pressure Ejection, John Wiley, New York.

Todd, A.J. and Millar, J. (1983) Receptive fields and responses to ionophoretically applied noradrenaline and 5-hydroxytryptamnie of units recorded in laminae I-III of cat dorsal horn, Brain Res., 288: 159–167.

Verberne, A.J.M., Owens, N.C. and Jackman, G.P. (1995) A simple and reliable method for construction of parallel multibarreled microelectrodes, Brain Res. Bull., 36: 107–108.