ELSEVIER

Journal of Neuroscience Methods 72 (1997) 1–4

JOURNAL OF NEUROSCIENCE METHODS

A simple method for the construction of a recording-injection microelectrode with glass-insulated microwire

M.L. Tsai[a,b], C.Y. Chai[a], C.-T. Yen[b,*]

[a]*Institute of Biomedical Sciences, Academica Sinica, Taipei, Taiwan, ROC*
[b]*Department of Zoology, National Taiwan University, # 1, Section 4, Roosevelt Road, Taipei, Taiwan, 10764, ROC*

Received 22 February 1996; revised 16 July 1996; accepted 25 July 1996

Abstract

A rapid method for the production of a glass-insulated microwire electrode is described. A microwire was threaded into a glass capillary which was then pulled on a vertical pipette puller. A conical tip of the microwire was formed when the strongly heated glass capillary broke together with the wire in it. A tight seal of the glass-insulated microwire electrode between the glass and the metal was accomplished with silicone glue. The manufactured electrode performed consistently at different immersion depths, and yielded stable recordings of single units in the cerebral cortex and the medulla of rats. The strength and low impedance characteristics of the glass-insulated microwire electrode may make it useful for the recording of single units in deep brain structures. Furthermore, the electrode can be easily combined with another glass micropipette to form a dual recording-injection microelectrode unit. © 1997 Elsevier Science B.V.

Keywords: Injection; Metal microelectrode; Microwire; Single unit recording

1. Introduction

Microinjection of chemicals into brain tissue has been routinely used to investigate and characterize the functions of specific groups of neurons within the brain. Frequently, it is desirable to record single unit activity at the same locus so that input-output characteristics are assessed simultaneously (McAllen and Spyer, 1978; Adams and Foote, 1988; Gottschaldt et al., 1988). Metal microelectrodes offer several distinct advantages, including, ease of use, commercial availability and stable low noise recording, thus several types of recording-injection microelectrodes have been developed with the use of a metal microelectrode (Fries and Zieglgänsberger, 1974; Adams and Foote, 1988; Gottschaldt et al., 1988; Hellier et al., 1990; Godwin, 1993; Haidarliu et al., 1995; Li et al., 1995). Most of these microelectrodes have been designed specifically for iontophoresis. The microelectrode combined with a needle, however, cannot accurately eject fluid in quantities less than 100 nl (Adams and Foote, 1988).

The tungsten microelectrode/micropipette pairs described by Fries and Zieglgänsberger (1974) and Li et al. (1995) are suitable for delivering fluid in quantities in the nanoliter range. Fries and Zieglgänsberger (1974) inserted an electrolytically sharpened tungsten wire into one of the compartments of a theta capillary. Li et al. (1995) made a tungsten-in-glass microelectrode by collapsing a glass tube onto a tungsten wire and then etching the glass-sheathed tungsten wire. Delicate maneuver is needed to produce these tungsten microelectrode/micropipette pairs. Furthermore, strong acid or base are needed in both cases for the etching process which may not be desirable for many electrophysiology laboratories.

Low noise, stable single unit recording can also be obtained with microwire, which has become widely

* Corresponding author. Tel.: +886 2 3630231, ext. 3322; Fax: +886 2 3636837.

PII S0165-0270(96)02151-6

available in recent years. Utilizing the microwire, we designed a rapid method for manufacturing metal-in-glass microelectrode. Because of its thin and slender shank, this metal-in-glass microelectrode can be easily combined with a microinjection capillary to make a recording-injection microelectrode.

2. Methods and results

Microwires, approximately 30 μm in diameter, were used. They were the fine strand stainless-steel microwires extracted from biomedical wire AS 634, Cooner Wire (Chatsworth, CA). A segment of the microwire was cut about 100 mm in length and threaded through a commercially available glass capillary (such as # 6260, A-M systems). The resulting capillaries were pulled on a vertical micropipette puller. The exact heat intensity and pull strength depended upon the type of puller used. To pull micropipettes with long and slender shank, seven glass capillaries were clamped in a bundle and pulled together with the magnetic dial set to zero and the intensity dial set to 18 A on a Narishige PE-2 puller (Tokyo). Both the glass and microwire were strongly heated and broken in one process. A total of 14 metal-in-glass microelectrodes were formed. Most of the microelectrodes had a shank diameter of about 150 μm, 15 mm from the tip.

The tip of the pulled microwire had a conical shape (Fig. 1C) and was usually covered with excess glass. Working under a microscope, the excess glass was snapped off with a fine forceps. A small drop of silicone rubber glue (Wacker Silgel 604) was applied to the shoulder of the metal-in-glass microelectrode. The open space between the glass and the microwire would be filled with the silicone rubber glue by capillary action. Each microelectrode was baked in an oven for 15–20 min at 80–90°C. Excess glue over the tip was removed by briskly and lightly rubbing the glass capillary from stem to tip with the thumb and the forefinger of the experimenter. The microelectrode was completed when the free end of the microwire was soldered to a suitable connector for the headstage of the preamplifier.

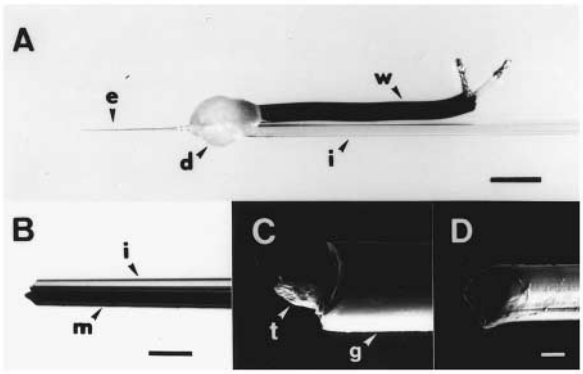

Fig. 1. Photomicrograph of a complete recording/injection electrode with connecting wire and shielding. A 30 μm diameter stainless steel wire was used. A. Full picture of the electrode pair. d, Dental cement; e, recording/injection microelectrode; i, injection micropipette; w, electrode wire (welded to the microwire) with shielding. Calibration bar, 5 mm. B. Enlarged view of the tip of recording/injection microelectrode in (A). i, injection micropipette; m, metal microelectrode. Calibration bar, 100 μm. C. Scanning electron micrograph of the tip of a representative glass-insulated microwire. t, tip of the microwire; g, insulation glass. D. Scanning electron micrograph of the tip of the microwire cut with a pair of sharp scissors. Calibration bar for (C) and (D), 10 μm.

The impedance of nine such microelectrodes, measured from the voltage drop of a 1 kHz, 400 mV sine wave source passing through a serially-connected 1 MΩ resistor, varied from 0.5 to 1.6 MΩ (mean 1.0 MΩ). In addition, the impedance scarcely changed ($< 0.1\%$), when the immersion depth in the saline solution was increased from 1 mm to 5 mm.

The metal-in-glass microelectrode was slender and straight, thus it could be easily combined with another glass capillary. We prepared such recording/injection microelectrodes as follows. A short length (approximately 10 mm) of the metal-in-glass microelectrode was cut. The optimal length depended on the length and the profile of the injection micropipette. The two glass capillaries fit best when the metal-in-glass microelectrode was shorter than the shank of the injection micropipette. A capillary micropipette was pulled and cut to the desired diameter (Briano, 1983). It was placed on a plasticine pedestal affixed to a microscopic slide. Under a stereomicroscope, a tiny droplet of freshly mixed epoxy was applied to the shank of the micropipette 5–10 mm from the tip. The prepared metal-in-glass microelectrode was positioned against the micropipette, and the relative position of the tips of the micropipette and metal-in-glass microelectrode adjusted accordingly. By means of capillary action, the epoxy glue filled the entire contact surface of the two glass capillaries, and set within 10 min.

A picture of a complete recording/injection microelectrode is shown in Fig. 1. These hybrid microelectrodes were used to record the effects on adjacent single unit by sodium glutamate solution ejected via the micropipette. One result is shown in Fig. 2.

3. Discussion

The microwire-in-glass microelectrode described has the following advantages; (1) all materials used were commercially available, (2) no corrosive base or acid was required to manufacture the microelectrode, and (3) sharp tip was formed in one step. No additional modification was necessary.

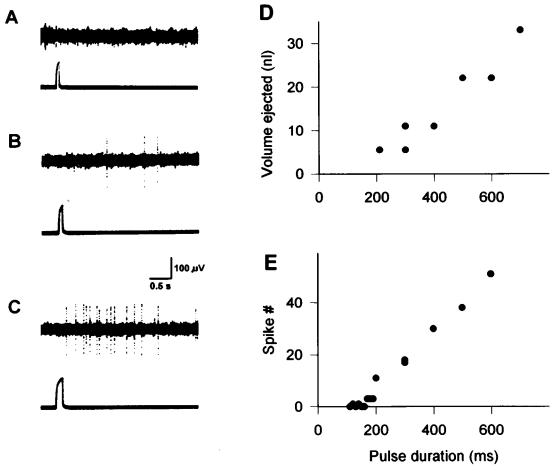

Fig. 2. An example of single unit activities recorded in the ventrolateral medulla of the rat with the glass-coated stainless steel microwire in the recording/injection electrode pair. 10 mM sodium glutamate solution (in artificial cerebrospinal fluid) was injected via the micropipette. (A), (B) and (C) are examples of original tracings of the unit's responses (upper trace) to different injection pulse durations (lower trace). (A) 130 ms; (B) 160 ms; (C) 200 ms. Injection pressure was 20 psi. (D) The volumes injected plotted against pulse durations. Volumes injected were calculated by $\pi r^2 \times h$. Where r was the radius of the inner cross section area of the micropipette and h was the height of the fluid column injected. h was measured under a stereomicroscope ($25\times$) of the distance travelled by the meniscus of the top surface of the fluid. (E) Dose-response curve of the number of spikes evoked by different pulse durations. The same set of data was used in (A), (B), (C), (D) and (E).

The present paper describes a new method for preparing a microwire for deep tissue recording. Many other techniques are currently available. The wires can be bundled together and cut (McNaughton et al., 1983). This produces a blunt tip with spaces between the wires. The wire, or wire bundle, can be inserted into a guiding cannula (Diana et al., 1987; Wiener, 1993) or attached to a guide post. These electrodes are relatively large and more likely to cause tissue damage. Elaborate technique has been developed to ensheath a microwire in quartz glass and subsequently grind it on diamond dust to produce a straight and sharpened tip (Krüger, 1982). By pulling a microwire taut with a rubber tube mechanism, microwires as thin as 80 μm can be advanced in soft tissue (Eckhorn and Thomas, 1993). These methods, however, require technical expertise beyond that found in an ordinary electrophysiology laboratory. Although the microelectrode described here is not as sharp as those made by Krüger, it is much easier to make and it records well. On the other hand, the conical tip of our electrode will penetrate soft tissue more easily than an electrode bundle or an electrode cannula.

As indicated earlier, our main purpose was to produce a recording/injection microelectrode pair. The microelectrode should be easy to prepare. It should be able to record stable single unit activities, at the same time, be able to deliver an exact amount of solution in the range of several to tens of nanolitres from a known distance, the closer, the better. This we accomplished in the microelectrode pair constructed in the present work. One may ask whether it would be even easier to glue a piece of microwire directly to a glass micropipette? We found this design unsatisfactory. As shown in Fig. 1(d), the cut end of the microwire is blunt and it usually curves slightly. Furthermore, gluing the flexible microwire, either formvar or teflon insulated, onto the glass micropipette presented some difficulties. It was hard to keep the wire straight during the gluing and adjusting process. It was necessary to use more epoxy glue. The shank portion of the electrode pair becomes bulky near the tip. In contrast, the sturdy and smooth surface of the glass capillary of our microelectrodes permitted the glue to permeate evenly along the entire length of the shank. In addition, the relative positions of the microelectrode and micropipette can be adjusted easily. A sharp and slender hybrid recording/injection microelectrode can be formed.

In summary, we developed a simple technique to ensheath a microwire in a slender glass capillary. The ease of combining this metal microelectrode with an injection micropipette made it especially useful for studying of neurally controlled functions where the inertia of the target tissue in the central nervous system is too great for iontophoresis injection to yield any noticeable functional change.

Acknowledgements

The authors thank C.H. Cheng for assistance making scanning electron micrographs. We thank W.C. Wu, R.J. Chiou and F.Z. Shaw for technical assistance. We thank A.F. Warneke for reading the manuscript. This work was supported by National Science Council, R.O.C. under contract No. NSC 84-2331-B002-153-M04.

References

Adams, L.M. and Foote, S.L. (1988) Effects of locally infused pharmacological agents on spontaneous and sensory-evoked activity of locus coeruleus neurons, Brain Res. Bull., 21: 395–400.

Briano R.A. Jr. (1983) A reproducible technique for breaking glass micropipettes over a wide range of tip diameters, J. Neurosci. Methods, 9: 31–34.

Diana, M., Garcia-Munoz, M. and Freed, C.R. (1987) Wire electrodes for chronic single unit recording of dopamine cells in substantia nigra pars compacta of awake rats, J. Neurosci. Methods, 21: 71–79.

Eckhorn, R. and Thomas, U. (1993) A new method for the insertion of multiple microprobes into neural and muscular tissue, including fiber electrodes, fine wires, needles and microsensors, J. Neurosci. Methods, 49: 175–179.

Fries, W. and Zieglgänsberger, W. (1974) A method to discriminate axonal from cell body activity and to analyse silent cells, Exp. Brain Res., 21: 441–445.

Godwin, D.W. (1993) A tungsten-in-glass iontophoresis assembly for studying input-output relationships in central neurons, J. Neurosci. Methods, 49: 211–223.

Gottschaldt, K.-M., Hicks, T.P. and Vahle-Hinz, C. (1988) A combined recording and microiontophoresis technique for input-output analysis of single neurons in the mammalian CNS, J. Neurosci. Methods, 23: 233–239.

Haidarliu, S., Shulz, D. and Ahissar, E. (1995) A multi-electrode array for combined microiontophoresis and multiple single-unit recording, J. Neurosci. Methods, 56: 125–131.

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

Krüger, J. (1982) A 12-fold microelectrode for recording from vertically aligned cortical neurones. J. Neurosci. Methods, 6: 347–350.

Li, C.Y., Xu, X.Z. and Tigwell, D. (1995) A single and comprehensive method for the construction, repair and recycling of single and double tungsten microelectrodes, J. Neurosci. Methods, 57: 217–220.

McAllen, R.M. and Spyer, K.M. (1978) Two types of vagal preganglionic motoneurones projecting to the heart and lungs, J. Physiol., 282: 353–364.

McNaughton, B.L., O'Keefe, J. and Barnes, C.A. (1983) The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records, J. Neurosci. Methods, 8: 391–397.

Wiener, S.I. (1993) Spatial and behavioral correlates of striatal neurons in rats performing a self-initiated navigation task, J. Neurosci., 13(9): 3802–3817.