Neuroscience Research, 8 (1990) 214–219
Elsevier Scientific Publishers Ireland Ltd.

NEURES 00348

Multibarreled glass-coated tungsten microelectrode for both neuronal activity recording and iontophoresis in monkeys

Bao-Ming Li *, Zhen-Tong Mei * and Kisou Kubota

Department of Neurophysiology, Primate Research Institute, Kyoto University, Inuyama City, Aichi (Japan)

(Received 1 March 1990; Accepted 14 March 1990)

Key words: Tungsten microelectrode; Extracellular recording; Iontophoresis; Frontal cortex; Behaving monkey

SUMMARY

A multibarreled glass-coated tungsten microelectrode suitable for single-unit recording and iontophoresis in chronically behaving animals is described. The microelectrode was stiff enough to pass through the dura matter of behaving monkeys, and can be applied as a microiontophoretic electrode. The electrode is easy to build and usable in stabilized conditions after repeated penetrations.

The multibarreled glass microelectrode with carbon fiber inside the central barrel [1,4,6] has been successfully used for single-unit recording and iontophoresis in the frontal cortex of behaving monkeys and in other cortical areas of other species. Since it allows marking of the recorded sites with carbon-fiber deposits (less than 100 μm in size), the estimate of laminar locations of drug-sensitive neurons or task-related neurons becomes possible with a discrepancy of less than 10% [5–7]. However, this electrode has one technical disadvantage in that it cannot penetrate through the dura matter into the cortex. It is necessary to anesthetize the monkey, drill a hole in the skull, and incise the dura to expose the cortex so that the electrode can penetrate under direct vision of the cortex.

We have succeeded in producing an easy-to-build multibarreled glass-coated tungsten microelectrode so that the disadvantage of the multibarreled carbon-fiber glass microelectrode can be overcome and the advantages of both tungsten-in-glass microelectrodes and multibarreled glass micropipettes could be combined. The multibarreled glass-coated tungsten microelectrode has been used in neuronal activity recording and drug iontophoresis in the frontal cortex of behaving monkeys performing a visual discrimination task with GO/NO-GO performance, and proved to function quite well.

Construction of the electrode Tungsten wire (0.3 mm diameter, 7–8 cm length: Nihon Tungsten Co., Tokyo) was used for the metal recording electrode. The coating tube

* *Present address*: Shanghai Institute of Physiology, Chinese Academy of Sciences, Yue Yang Road 320, Shanghai, People's Republic of China.
Correspondence: Kisou Kubota, M.D., Dept. of Neurophysiology, Primate Research Institute, Kyoto University, Inuyama, Aichi 484, Japan. *Tel.* 0568-62-9551; *Fax*: 0568-62-2428.

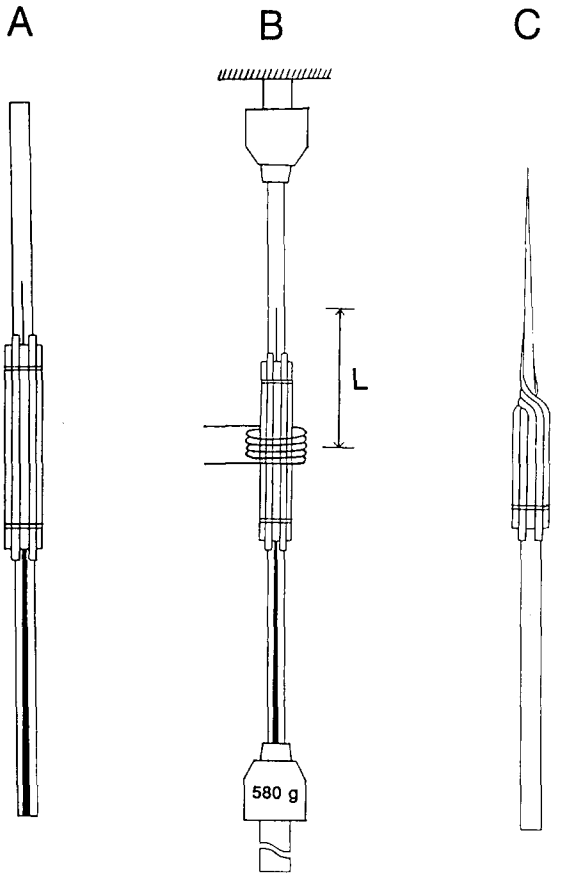

Fig. 1. (A) A core tube with a tungsten microelectrode inside and 3–5 external tubes were bundled at the middle portion of the tungsten-containing core tube with stainless wires. (B) For pulling, the bundle was placed centrally in the heating coil. The length from the tip of the tungsten microelectrode to the center of the heating coil (L) determined the length of the multibarreled electrode array. (C) Outline of a multibarreled glass-coated tungsten microelectrode.

(C-tube) was a glass capillary 2 mm in outer diameter and 10 cm in length. The iontophoretic tube (I-tube) was a glass capillary 1 mm in outer diameter and 3–4 cm in length. Both C- and I-tubes contained a glass filament, and were pulled by the authors from standard Pyrex glass tubes (7 mm outer diameter, 5 mm inner diameter, Summit Medical Co., Tokyo) with a glass-capillary puller (Type C-555, Summit Medical Co., Tokyo). At first, one end of the tungsten wire was corroded in 10% NaOH solution by anodal DC current (10–15 V, 0.3–1.0 A). The corroded portion of the tungsten wire was sharpened gradually so that the diameters at the tip, at 100 μm from the tip and 1 mm from the tip were 1–3, 20–30 and 80–100 μm, respectively. Thereafter, the tungsten wire was inserted into the C-tube. Four to 6 I-tubes were bundled at the middle portion of the tungsten-containing C-tube with stainless wire (Fig. 1A). The bundle was then pulled down by a microelectrode puller (Type PE-2, Narishige, Tokyo), using proper parameters of heating current (20–22 A).

Pulling the microelectrode was a two-stage process. In the first stage, the bundle was fixed centrally in the heating coil of the puller (Fig. 1B). The heating coil was a 5-round coil 6 mm in height and 9 mm inner diameter, prepared by the authors from a nichrome

wire 1 mm in diameter supplied by the puller maker (Narishige, Tokyo). The heat was then applied, while the bundle was held with one hand. When sufficiently molten, the bundle was twisted by hand *anticlockwise by 360–450 degrees* (Fig. 1C). After twisting, the heat was turned off, and the bundle was then cooled. In the second stage, the heat was applied again, and lengthening was allowed to occur naturally by gravitation (580 g) without removal of the heat until the multibarreled glass-coated tungsten microelectrode was formed. Figure 1C shows schematically the outline of the electrode; Figure 2 shows microphotographs of the electrode tip before and after a single penetration.

The Narishige PE-2 microelectrode puller, originally designed for the pulling of a glass micropipette, had 3 switches which were switched off automatically when the molten glass bundle was lengthened to the extent that the heat of the heating coil could be turned off and the magnet force for pulling could be added. For pulling our microelectrode, the puller was modified. The 3 switches were prevented from turning off during the entire process of lengthening the molten glass bundle by fixing them with gum-rings so that lengthening 'without removal of the heat' could be realized, magnet force for pulling could not be started, and only 580 g weight could act as the pulling force. This modification was found to be of crucial importance in constructing a stabilized and reliable electrode.

Physical and electrical characteristics The shape of the multibarreled glass-coated tungsten microelectrode is shown in Figure 2. An exposed tip of the tungsten microelectrode had a 1–3 μm diameter and 5–30 μm length, with which neuronal activity recording and drug iontophoresis could be successfully carried out. The length of the twisted portion could be controlled within a range of 1.5–5 cm by putting the bundle in the heating coil of the puller so that the tungsten tip inside the C-tube was located at a required length (L) above the heating coil (Fig. 1B). The diameter, 3 cm from the tip, was about 1.0 mm. In general, the impedances of electrodes with exposed tungsten tip of 10–15 μm were 4–5 MΩ (an AC constant current source, 20 Hz, 0.02 μA). To investigate the effects of DC iontophoretic current passed through the barreled glass micropipette on the resistance of the glass-coated tungsten microelectrode, we inserted the electrode into 0.9% saline solution, passed the iontophoretic current (100 nA) through the barreled micropipette ejected with 0.9% saline, and simultaneously measured the impedance of the tungsten microelectrode in the C-tube. The result showed that iontophoretic current had no measurable effects on the impedance of the tungsten microelectrode.

Neuronal activity recording and iontophoresis For neuronal activity recording and drug iontophoresis, the tungsten microelectrode coated in the C-tube was connected via a silver wire to the input of a high-input impedance amplifier. One of the I-tubes was filled with 0.9% saline to balance the iontophoresis, while the others were filled with drugs and connected via silver wire to an iontophoresis unit (Type DP-30a, Dia Medical System Co., Tokyo).

Recordings were carried out for the frontal cortex of a behaving monkey whose head was rigidly fixed to the stereotaxic frame of a custom-made monkey chair. The multibarreled glass-coated tungsten microelectrode was inserted across the dura into the cortex by a pulse-motor-driven micromanipulator (Type ME-71, Narishige, Tokyo) and gently advanced by the manipulator to obtain neuronal activities, while the monkey was performing a task. When task-related neuronal activity appeared, drugs were iontophoresed onto the task-related neuron to investigate the effects of the drugs on the task-related activity. Iontophoretic current was usually 10–60 nA and the current polarity depended on the drugs. An example of an extracellular recording of neuronal spikes

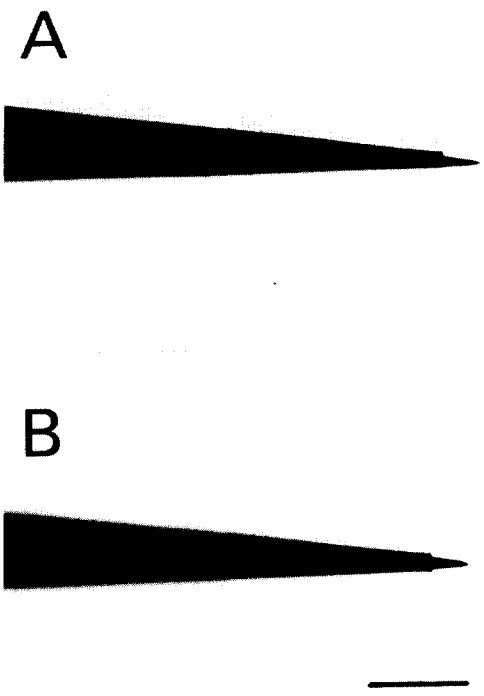

Fig. 2. Microphotographs showing the tip of a multibarreled glass-coated tungsten microelectrode before (A) and after penetration (B). The tip of the electrode was not destroyed after penetrating the dura into brain tissues. Scale bar = 50 μm.

obtained with the multibarreled glass-coated tungsten microelectrode in the frontal cortex of a chronically behaving monkey is shown in Figure 3.

Figure 3 shows spontaneous neuronal activities recorded with a 3-barreled glass-coated tungsten microelectrode in the premotor cortex of a behaving monkey. One barrel (I-tube) of the microelectrode contained 10 mM bicuculline methiodide (BMI, Sigma, U.S.A.) solution. Two barrels contained 0.9% saline solution, one of which was for iontophoretic balance and the other for control testing of the iontophoretic current. The exposed tungsten tip of the microelectrode was 18 μm in length and 2 μm in tip diameter. The upper traces in Figure 3 (A, B, C) show spontaneous discharges before, during the iontophoretic application of BMI and after the end of the application of BMI, respectively, whereas the lower traces show superimposed spikes before and during the application of BMI and after the end of the application, respectively. Three units appear to be present in this recording (see the spike superimpositions in B and C). BMI, applied with 15 nA, significantly increased the discharge rate of the unit with the largest amplitude. It can be seen that the spike outline and amplitude were kept the same before and during the application of BMI. Thus the iontophoretic current showed no effect on the recording. Furthermore, the control current of 15 nA had no effect on the discharging rate of the unit (not shown).

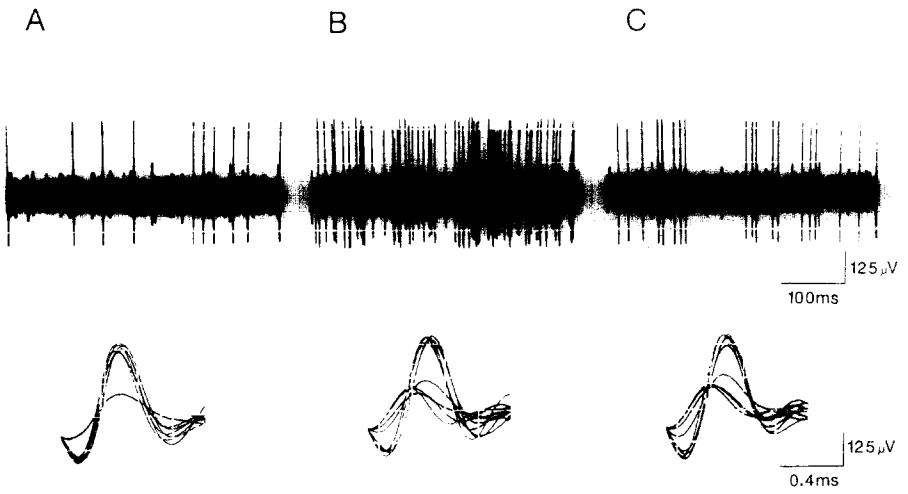

Fig. 3. Spontaneous neuronal activities recorded with a 3-barreled glass-coated tungsten microelectrode in the premotor cortex of a behaving monkey. (A) Discharges before the application of BMI. (B) Discharges during the application of BMI (15 nA), sampled about 15 s after BMI iontophoresis began. (C) Discharges sampled about 3 min after the end of BMI iontophoresis. The lower traces in A, B and C show superimposed spikes before and during the application of BMI and after the end of the application of BMI, respectively. There were 3 units in this recording (see lower traces in B and C). Iontophoretically applied BMI significantly increased the discharge rate of the neuron with the largest amplitude, whereas its spike shape was not distorted by the iontophoretic current.

The neuronal activity recorded with the electrode could last stably for several hours if the monkey did not move excessively. After each recording, we checked the tip of the electrode under the microscope. Except for a small amount of tissue-like debris which sometimes accumulated at the tip, we did not find any serious penetration damage of the glass-pipette, even if the dura was thickened by connective tissues 3–4 months after microsurgery during which a small hole was drilled in the skull and the dura exposed. Thus, it was almost 100% successful in obtaining reliable single-unit recordings and iontophoresis. In our experiments, one electrode was usually used only for one session of neuronal activity recording and drug iontophoresis.

We have described a multibarreled glass-coated tungsten microelectrode suitable for both neuronal activity recording and drug iontophoresis in behaving monkeys. Because the tungsten wire is used, the electrode becomes stiff enough to be inserted easily across relatively tough and thickened dura of behaving monkeys without any damage to the barreled micropipette. Drugs can be applied iontophoretically onto neurons being recorded through the barreled micropipette, and iontophoretic current exerts no effect on the electrical properties of the electrode and therefore does not distort recorded spikes. The multibarreled glass-coated tungsten microelectrode can easily be made in laboratories equipped to produce a single tungsten electrode and glass micropipette.

A multibarreled electrode array containing an Elgiloy microelectrode has been used in orbitofrontal studies [2,3]. In this electrode array, the glass micropipette was "glued' to a glass-coated Elgiloy microelectrode. Technically, it may be easier to construct a bundled array rather than a 'glued' array.

The multibarreled glass-coated tungsten microelectrode has a disadvantage in that it cannot mark the recorded sites in monkeys as does the multibarreled carbon fiber

microelectrode. Elgiloy microelectrodes have been demonstrated to be effective in marking recorded sites [8,9], but so far we have not succeeded in constructing a multibarreled microelectrode array containing an Elgiloy.

The shape of the corroded tungsten wire tip is an important factor in producing the multibarreled tungsten microelectrode array. The gradual sharpening of the tungsten microelectrode appears important. For the pulling of the multibarreled glass-coated tungsten microelectrode, we modified the Narishige PE-2 glass micropippette puller so that the apparatus had only the functions of an AC current supply for heating the coil and a puller of 580 g weight for pulling the molten bundle. This modification seems to be another important factor in constructing the multibarreled glass-coated tungsten microelectrode.

ACKNOWLEDGEMENTS

We would like to thank Drs. M. Matsumura and A. Mikami for their helpful suggestions. We are also grateful to Mrs. T. Miwa and Mrs. K. Watanabe-Sawaguchi for their technical assistance.

REFERENCES

1 Armstrong-James, M. and Millar, J., Carbon fiber microelectrodes, *J. Neurosci. Meth.*, 1 (1979) 279–287.
2 Aou, S., Nishino, H., Inokuchi, A. and Mizuno, Y., Influence of catecholamines on reward-related neuronal activity in orbitofrontal cortex, *Brain Res.*, 267 (1983) 165–170.
3 Aou, S., Oomura, Y. and Nishino, H., Influence of acetylcholine on neuronal activity in monkey orbitofrontal cortex during bar press feeding task, *Brain Res.*, 275 (1983) 178–182.
4 Fox, K., Armstrong-James, M. and Millar, J., The electrical characteristics of carbon fiber microelectrodes, *J. Neurosci. Meth.*, 2 (1980) 37–48.
5 Sato, H., Fox, K. and Daw, N.W., Effect of electrical stimulation of locus coeruleus on the activity of neurons in the cat visual cortex, *J. Neurophysiol.*, 62 (1989) 946–958.
6 Sawaguchi, T., Matsumura, M. and Kubota, K., Long-lasting marks of extracellularly recorded sites by carbon fiber glass micropipettes in the frontal cortex of chronic monkeys, *J. Neurosci. Meth.*, 15 (1986) 341–348.
7 Sawaguchi, T., Matsumura, M. and Kubota, K., Depth distribution of neuronal activity related to a visual reaction time task in the monkey prefrontal cortex, *J. Neurophysiol.*, 61 (1989) 435–446.
8 Suzuki, H. and Azuma, M., A glass-insulated 'Elgiloy' microelectrode for recording unit activity in chronic monkey experiments, *Electroencephalogr. Clin. Neurophysiol.*, 41 (1976) 93–95.
9 Suzuki, H. and Azuma, M., A method for the accurate localization of recording sites in chronic monkey experiments. In M. Ito (Ed.), *Integrative Control Function of the Brain, Vol. 2*, Elsevier, Amsterdam, 1979, pp. 405–407.