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ARTICLE

Novel carbon fiber microeletrodes for extracellular electrophysiology

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ABSTRACT Single- and multibarrel carbon fiber microelectrode blanks were constructed and pulled to electrodes to be used for extracellular recording and microiontophoresis. A unique spark etching method was developed to produce a sharp-pointed, conical carbon tip protruding 15-20 μm from the glass pipette(s). The shape and size of the carbon fiber tip were examined by scanning electron microscopy. In test experiments, extracellular recordings were made from spinal dorsal horn neurons of the spinal cord in anesthetized rats. The sharp carbon tip allowed these electrodes to penetrate the arachnoid membrane over the spinal cord with ease. The electrodes picked up extracellular spikes with an excellent signal-to-noise ratio. Under the given experimental conditions, the peak-to-peak noise level was about 20 μV. To test the performance of the iontophoresis barrels, neurons were stimulated by iontophoretic application of *N*-methyl-D-aspartate (NMDA) or kainic acid or by noxious heat delivered to the cutaneous receptive fields in the tail. After the iontophoretic ejection of naloxone, the responses to iontophoresed kainic acid and noxious heat were significantly increased. Spikes from dorsal horn neurons were counted and peristimulus time histograms were displayed on-line by means of a LabView-based system. These carbon fiber microelectrodes are excellent for extracellular spike recording and microiontophoresis and may additionally be suitable for electrochemical measurements and for the development of enzyme- or antibody-based microbiosensors.

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KEY WORDS

multibarrel microelectrodes
carbon tip
spark etching
scanning electron microscopy
single-unit recording
microiontophoresis
electrochemistry
microbiosensors

Carbon fiber microelectrodes have been used to record extracellular action potentials since 1979 (Armstrong-James and Millar 1979; Armstrong-James et al. 1980a; Anderson and Cushman 1981). The carbon fibers are graphite monofilaments about 7 μm in diameter. In microelectrodes, they have good extracellular recording qualities similar to those of the best tungsten electrodes (Fox et al. 1980; Shigemitsu et al. 1980; Starrenburg and Burger 1982; Yavich 1998). These microelectrodes have been demonstrated to be suitable for in vivo electrochemical detection (Ponchon et al. 1979; Armstrong-James et al. 1980b; Millar et al. 1981; Stamford et al. 1984; Millar et al. 1992). Various voltammetric or amperometric techniques involving the use of carbon fiber microelectrodes have been developed for in vivo measurements of biogenic amines (Kruk et al. 1980; Buda et al. 1981; Cespuglio et al. 1981; Gonon et al. 1981; Akiyama et al. 1985; Rivot et al. 1987; Mermet et al. 1990; Kawagoe et al. 1991b; Suaud-Chagny et al. 1993; Rivot et al. 1995; Shigenaga et al. 1997; Daws et al. 1998) or nitric oxide (Malinski and Tah 1992; Yao et al. 1995; Friedemann et al. 1996; Park et al. 1998; Clarencon et al. 1999). Carbon fiber-based, enzyme-modified microbiosensors have been introduced for the in situ determination of glucose (Netchiporouk et al. 1996; Shram et al. 1997), acetylcholine (Kawagoe et al. 1991a; Navera et al. 1991; Tamiya and Karube 1992), choline (Garguilo and Michael 1996), lactate (Shram et al. 1998) or glutamate (Kulagina et al. 1999).

During the fabrication of such microelectrodes, individual carbon fibers can be inserted into borosilicate glass capillary tubing and single or multibarrel electrode blanks can easily be assembled. Because of the great tensile strength of the carbon fibers, they do not break when blanks are pulled to microelectrodes. After the pulling, the microelectrode is left with several centimeters of carbon fiber protruding from the glass tip. The simplest way to trim the end of the carbon fiber to the correct tip length (10-30 μm) is to cut off the excess with microscissors under a microscope. This is a difficult operation even for an experienced worker with steady hands, and the glass tip can easily be damaged. Another method of trimming the carbon fiber is electrochemical etching, applying dilute chromic acid (Millar and Williams 1988) or saline (Kuras and Gutmaniene 1995; Fu and Lorden 1996; Kuras and Gutmaniene 2000) and a few tenths of a mA of alternating current. A third technique is spark etching, which allows the best control of tip length and shape for selective

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extracellular unit recording or electrochemical measurements. In the present study we have developed a relatively simple method of carbon fiber microelectrode manufacturing, which uses spark etching to form a sharp-pointed conical tip with a length of 15-20 μm.

Materials and Methods

Manufacturing carbon fiber electrodes

Carbon fiber electrodes were fabricated from borosilicate glass capillary tubing obtained from World Precision Instruments (WPI, Sarasota, FL, USA). Single-barrel, recording-only carbon fiber electrodes were made from standard borosilicate glass tubing (1.50 mm o.d., 0.84 mm i.d.). Multibarrel recording/iontophoresis electrodes were constructed from the appropriate number of thin-wall glass tubes (1.50 mm o.d., 1.12 mm i.d.) glued together before pulling. The recording barrel contained no inner filament, whereas the iontophoresis barrels were made from glass tubing with a solid inner glass filament fused to the inner wall, which accelerates the filling of the barrels. A 15 cm long individual carbon fiber with a diameter of about 7 μm was glued to a 2.5 cm long 28 AWG tin-plated copper wire with conductive paint (Silver print, GC Electronics, Rockford, IL, USA). One end of the wire had previously been soldered into a gold-plated male connector pin. Beginning at its free end, the carbon fiber was sucked into the glass capillary tubing by gentle vacuum. The connector pin was then fixed onto the end of the glass tubing by 12 mm long heat-shrinkable plastic tubing. For single electrodes, this assembly was ready to be pulled. For multibarreled arrays, the appropriate number of inner filament-containing capillary tubes were attached to the recording barrel with two-component epoxy glue at both ends of the arrays. The glued portions of the arrays were covered with 15 mm long heat-shrinkable plastic tubing at both ends to provide further stability for the arrangement and suitable locations for keeping the multibarrels in place during pulling and in the electrode holder.

The two ends of the electrode "blank" were then held by the chucks of the vertical electrode puller (PE-2, Narishige Scientific, Tokyo, Japan) and a heating coil was used to melt the glass gently in the central portion of the assembly. As the glass was beginning to soften, the lower chuck was slowly rotated by one-half to two-thirds of a full circle while, the electrode blank was pulled slowly by gravity only. This rotation and pulling caused the lengths of tubing to fuse together. The combination of the current supply to the heating coil and the degree and timing of the pull may be varied to produce pipettes of different lengths and diameters. In consequence of the very high tensile strength of the carbon fiber, it did not break during the pulling procedure. The excess fiber protruding from the tip of the glass assembly was shortened with fine scissors to about 5 mm. The exposed carbon fiber was finally trimmed by spark etching (Millar 1992; Williams et al. 1992a,b) under a light microscope. Sparks were generated by a high voltage of about 800 V, with a piece of polished gold wire as counter electrode. Finally, the open ends of the glass tubing were heated up and bent out radially from the center to facilitate access and to reduce cross-contamination between barrels during filling.

Scanning electron microscopy

Electrode samples for scanning electron microscopy were coated with conductive films of gold with the aid of a sputter coater (SC-520, Bio-Rad, Hercules, CA, USA). Images of electrode tips were taken with a traditional scanning electron microscope (S-2400, Hitachi, Tokyo, Japan).

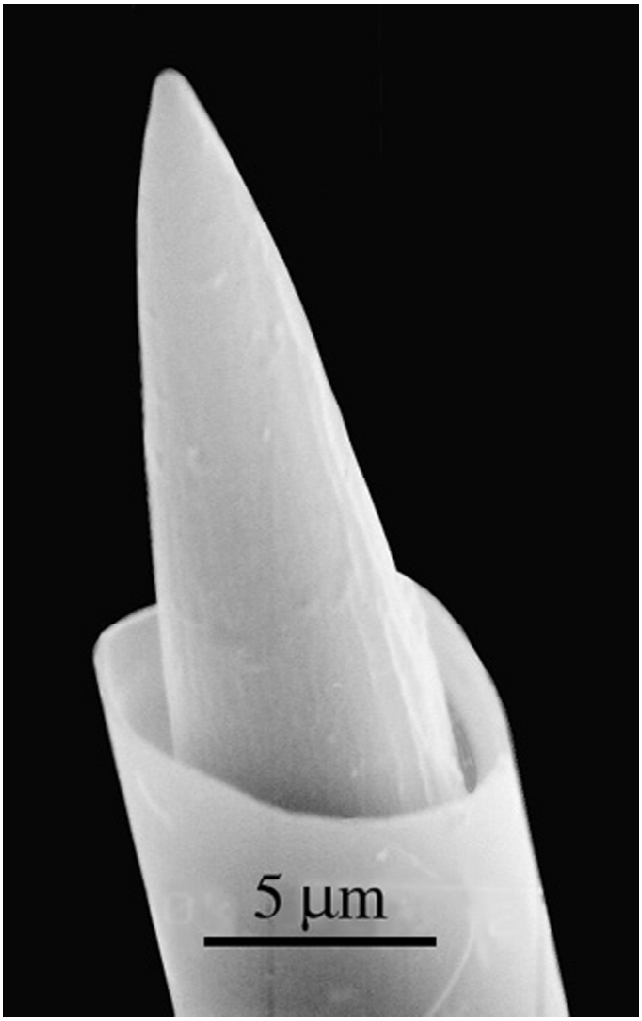

Figure 1. Scanning electron micrograph of the tip of a single-barrel carbon fiber electrode. The sharp-pointed, conical tip of the carbon fiber protrudes from the insulating borosilicate glass capillary tubing. The carbon tip is suitable for extracellular recording and for *in vivo* electrochemical measurements.

Anesthesia and surgery

Sprague-Dawley rats (300-400 g) of either sex were initially anesthetized with chloral hydrate (40 mg/100 g, i.p.). For single-unit recordings, the sacral spinal cord was exposed by a laminectomy and the rat was placed in a stereotaxic apparatus. The spinal cord was covered with a pool of warmed mineral oil. Body temperature was kept at 37°C by a water-heated blanket beneath the rat and an infrared heat lamp from above. Heart rate was monitored and maintained within normal limits for lightly anesthetized rats. Recordings were commenced not earlier than one hour after surgery. During the experiments, the animals were maintained in a lightly anesthetized state with additional i.p. injections of chloral hydrate, so that the rats showed no sign of discomfort, but the tail flick reflex could be evoked by the application of noxious heat (43-45°C) to the tail.

Extracellular recording

Extracellular, single-unit recordings were made from neurons of the dorsal horn of the sacral spinal cord. Recording/iontophoresis electrodes were constructed from a seven-barreled array of borosilicate glass capillary tubing as described above. Drug solutions were filled into the free ends of the iontophoresis barrels, and capillary action in the inner glass filaments ensured complete filling. The tip of the electrodes was kept in physiological saline during the filling procedure in order to prevent cross-contamination between the barrels and to prevent drying-out of the tip. Drugs were delivered iontophoretically using nanoampere currents of appropriate polarity. Action potentials were recorded with an ExAmp-20KB amplifier (Kation Scientific, Minneapolis, MN, USA) and displayed on an oscilloscope, and the activity of single units was isolated by using a window discriminator. The collection of experimental data and the iontophoretic delivery of drugs were automated by means of a multifunction instrument control and data acquisition board (PC-1200, National Instruments, Austin, TX, USA) programmed in

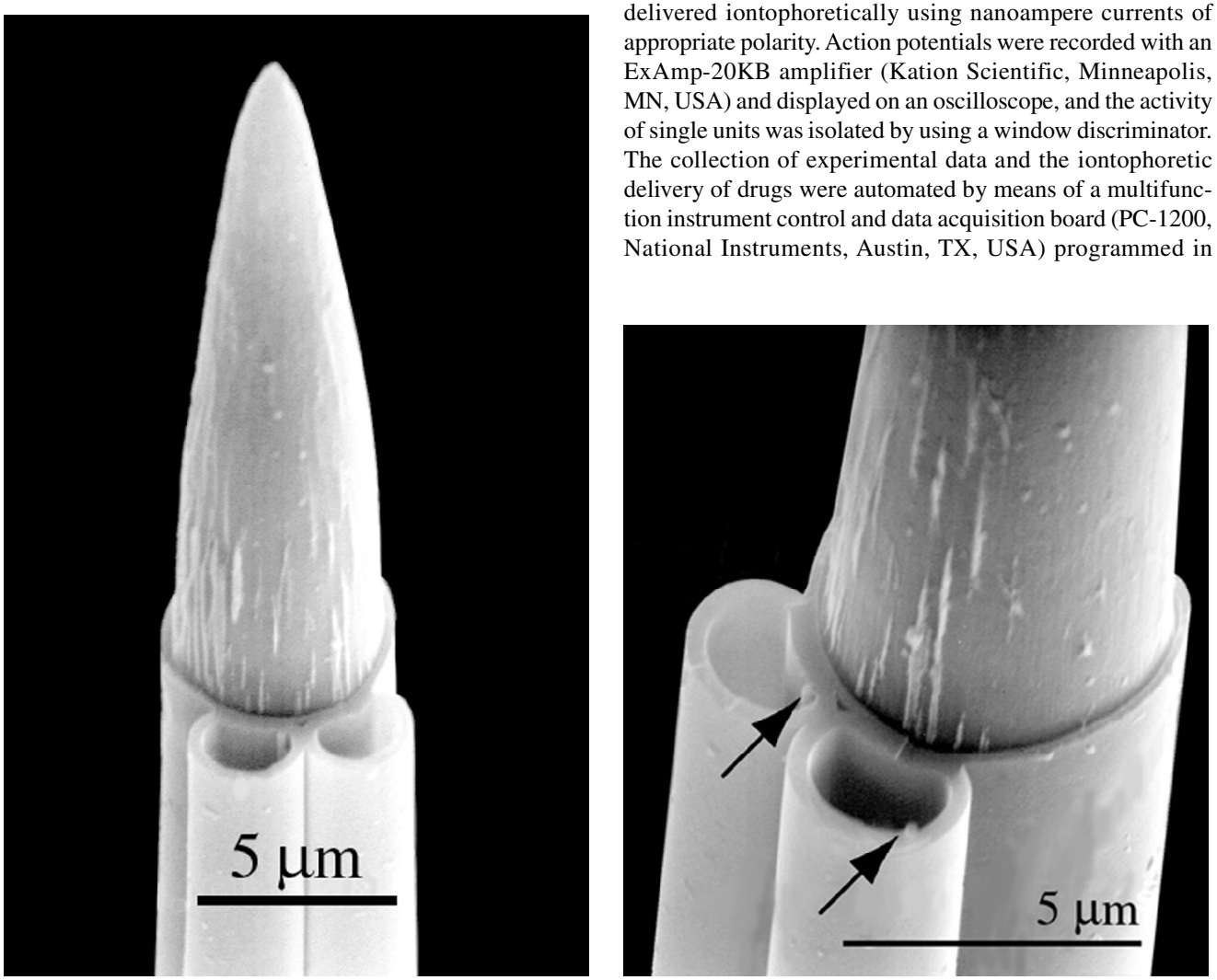

Figure 2. Fine structure of the tip of a three-barreled carbon fiber combination electrode as revealed by scanning electron microscopy. Left panel: the conducting carbon fiber tip records electrical signals; microiontophoresis can be performed through the microscopic orifices of the side-positioned iontophoresis barrels. Right panel: close-up view of the iontophoresis barrels. Note the internal glass filaments (arrows) that permit self-filling of aqueous solutions through the free ends of the capillary tubing.

LabView (National Instruments). LabView-based data acquisition and instrument control software was developed in-house; it has been published in part elsewhere (Budai 1994, 2000). Histograms of the neuronal activity were taken on-line by the computer-controlled system. Analog signals were sampled and digitized at 20 kHz by the data acquisition card, and oscilloscope trace-like recordings were taken from neurons at the beginning of each experiment.

Single-unit extracellular recordings were made from selected dorsal horn neurons responding to noxious heat delivered by a projector lamp focused on the blackened ventral surface of the tail. A thermistor probe placed in contact with the heated area was used to provide feedback control of the heat stimulus in order to prevent overheating. The temperature was raised to a peak of 50°C and held there for 5 s. Neurons were also characterized as low threshold (LT), nociceptive specific (NS) or wide dynamic range (WDR) according to their responses to mechanical stimuli of increasing strength. Both innocuous (brush, pressure) and noxious (pinch, squeeze felt to be painful by the experimenter) stimuli were applied to the excitatory receptive fields of the tail.

Microiontophoresis and drugs

Microiontophoresis was performed with Minion-16 iontophoresis current units (Kation Scientific, Minneapolis, MN, USA). The drug barrels of the combined seven-barreled recording/iontophoresis electrode contained one or other of the following freshly made solutions: 100 mM N-methyl-D-aspartate Na (NMDA) in 100 mM NaCl (pH 8.0), 20 mM kainic acid (KA) in 180 mM NaCl (pH 8.0), or 20 mM naloxone HCl in 180 mM NaCl (pH 5.0). NMDA and KA were delivered by negative currents, while naloxone was ejected by positive current. All drugs, including chloral hydrate, were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Results

Scanning electron microscopy

Scanning electron microscopy was used to inspect the shape and size of the carbon fiber tips trimmed by spark etching.

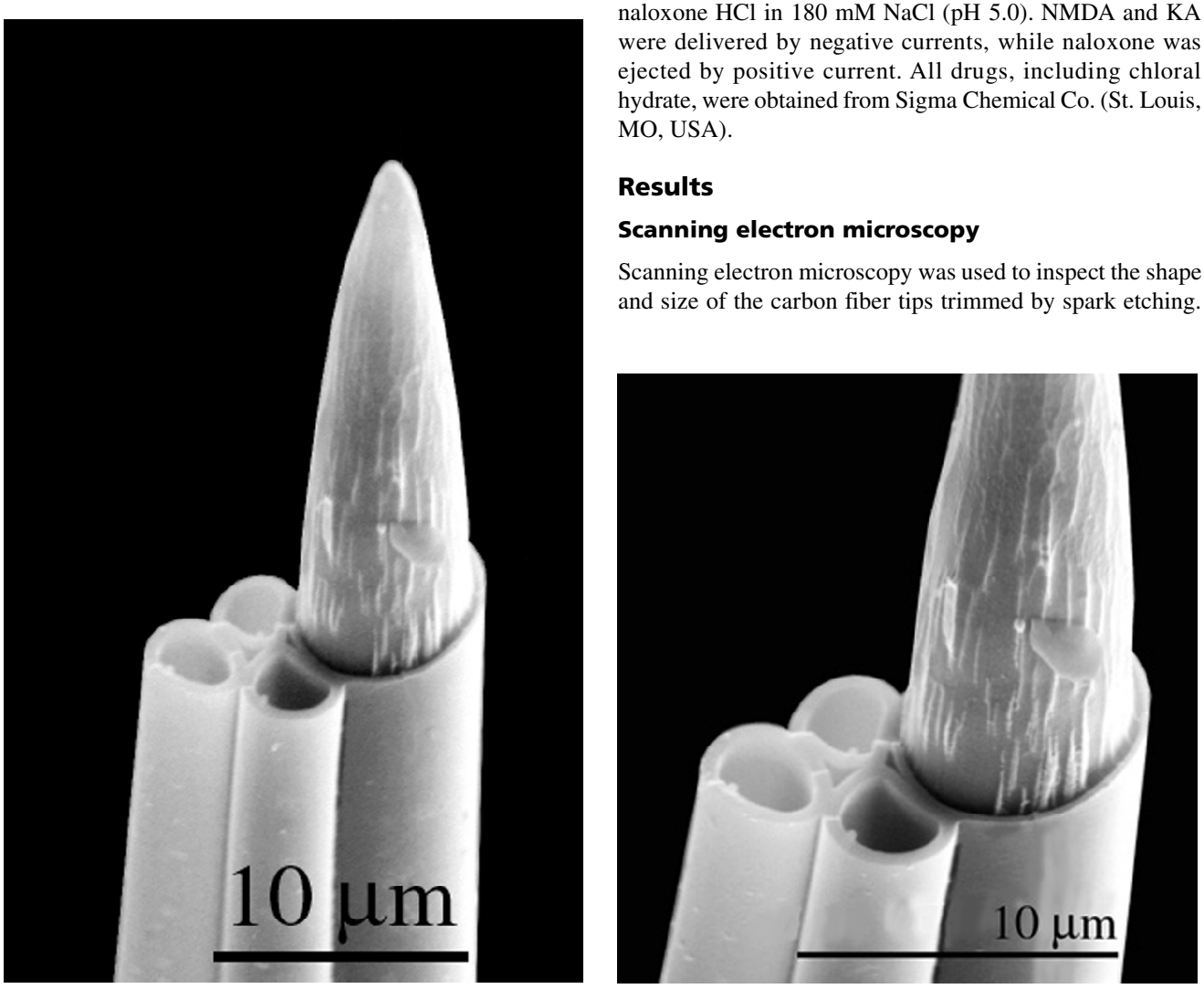

Figure 3. The four-barreled carbon fiber combination electrode allows the testing of three neuroactive compounds simultaneously. The carbon tip records extracellular spikes. Left panel: scanning electron microscopic image of the tip of a four-barreled electrode. Right panel: close-up view of the iontophoresis barrels.

As Figs. 1-4 show, this procedure resulted in characteristic, conical carbon tips that extended about 15 µm from the glass pipettes. Single-barrel electrodes were made for extracellular action potential recording and/or electrochemical measurements. The borosilicate glass wall around the carbon fiber provided the necessary electrical insulation (Fig. 1). In multibarrel assemblies, the tips of the iontophoresis barrels were very much smaller than the carbon fiber barrel. The spark etching of the carbon fiber in multibarrel microelectrodes was completed in the same way as for single electrodes, and their recording qualities were directly comparable with those of the single electrodes. Figure 2 shows a scanning electron micrograph of a typical three-barreled microelectrode. The glass insulation around the fiber ends is at the same level as the iontophoresis barrels. Three barrels are suitable when iontophoresis is used only to mark the recording site with iontophoresed tracer material or when one substance (or possibly two) is to be tested on neuronal firing. When a greater number of drugs are to be tested by microiontophoresis in combination with extracellular spike recording, four- or seven-barrel constructions should be used (Figs. 3 and 4, respectively). Electron microscopy revealed that in asymmetric arrangements of carbon fiber and iontophoresis barrels, such as in the three- and four-barrel electrodes, the iontophoresis barrels ended in oval or moon-shaped apertures (Figs. 2 and 3). In contrast, the symmetric construction of the seven-barrel electrodes resulted in six trapezoid-like iontophoresis orifices around the center barrel that contained the carbon fiber (Fig. 4). In some of the electrodes, however, the carbon fiber was pushed laterally away from the center position during the pulling process, which led to the formation of uneven iontophoresis openings (Fig. 4).

In vivo experiments

The recording quality of the carbon fibers and the performance of the microiontophoresis barrels were examined in experiments on anesthetized rats. Extracellular recordings were made from spinal dorsal horn neurons located between 100 and 600 µm from the dorsal surface of the spinal cord. The sharp carbon tip allowed these electrodes to penetrate the arachnoid membrane over the spinal cord with ease. In a few experiments, the electrodes successfully perforated even the dura mater over the rat spinal cord and extracellular recordings were possible. Measured in physiological saline at 1 kHz, the impedances of these electrodes lay between 400 KΩ and 1 MΩ. There was no consistent relationship between impedance and tip length. Within the nervous tissue, the electrodes picked up extracellular spikes with an excellent signal-to-noise ratio. Under the experimental conditions applied, when 30 Hz to 8 KHz filters were used, the peak-

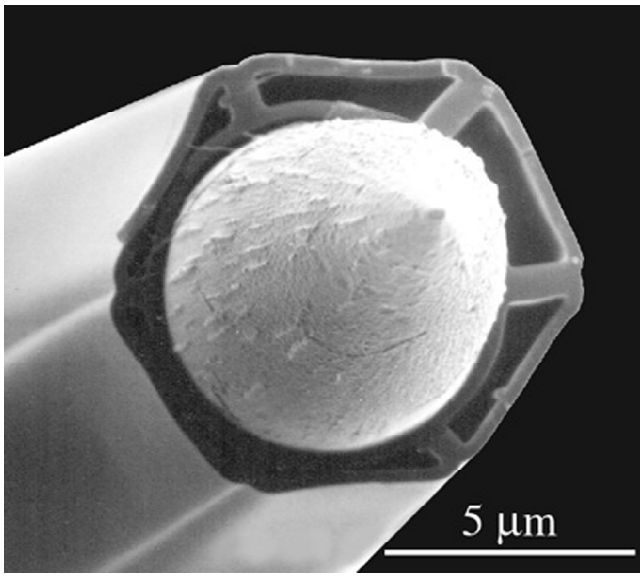

Figure 4. The seven-barreled carbon fiber combination electrode allows the testing of up to six neuroactive compounds on neuronal firing recorded by the carbon tip. Left panel: the fused-together glass micropipettes form a seal around the protruding carbon fiber. Right panel: close-up image of the iontophoresis barrels and the axial carbon fiber tip.

to-peak noise level was about 20 μV (Fig. 5). The impedances of iontophoresis barrels filled with 200 mM NaCl and measured at 1 kHz varied between 2 MΩ and 12 MΩ. The differences in impedance between the iontophoresis barrels of the seven-barreled electrodes were greater than those for the three- or four-barrel electrodes.

In sample experiments, the sensory neurons of the dorsal horn were stimulated by iontophoretic applications of NMDA and/or KA or by noxious heat delivered to the cutaneous receptive fields in the tail. Spike recordings and microiontophoresis of drugs were performed with seven-barreled carbon fiber electrodes. A sample record tracing of NMDA-evoked spikes is shown in Fig. 5. Spikes from dorsal horn neurons were counted by the data acquisition board and peristimulus time histograms were displayed on-line by means of the LabView-based system (Fig. 6). The effects of iontophoresed naloxone on the responses evoked by the application of iontophoresed NMDA or kainic acid or of peripheral noxious heat in a low threshold (LT) dorsal horn neuron are shown in Fig. 6. After the iontophoretic ejection of naloxone, the responses to iontophoresed kainic acid and noxious heat were significantly increased. In contrast, the responses to iontophoretically applied NMDA were markedly decreased (Fig. 6). With either single- or multibarreled carbon fiber electrodes, the quality of the spike recordings could be maintained for many hours.

Discussion

Single- or multibarrel carbon fiber microelectrodes are used to record extracellular action potentials. Extracellular "spikes" are typically a few hundred microvolts in amplitude and are generated by action potentials across the membranes of neurons. Extracellular recording of neuronal firing is often used in conjunction with the microiontophoresis of various neuroactive compounds (Hicks 1984). This requires compound recording/iontophoresis multibarrel microelectrode

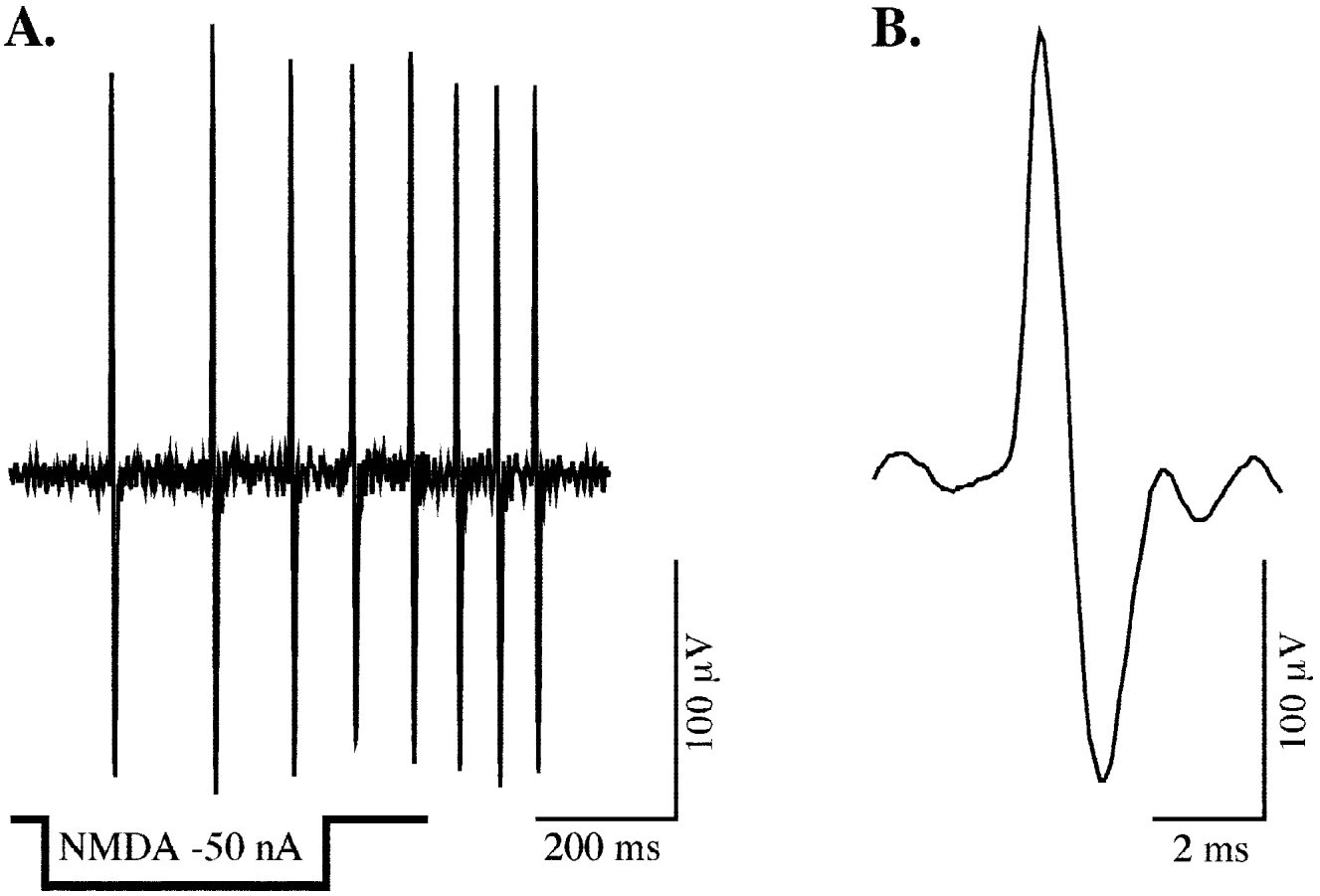

Figure 5. Sample spike records from a low threshold (LT) spinal dorsal horn neuron, using a seven-barreled carbon fiber recording/iontophoresis electrode. **A.** Neuronal firing was evoked by NMDA iontophoresis as shown. **B.** Shape of an individual extracellular action potential. Note the excellent signal-to-noise ratio recording achieved with the carbon fiber electrode.

assemblies capable both of leading electrical signals into the preamplifier and of delivering test compounds into the near vicinity of neurons, using electrical currents in the nanoampere range. The basic multibarrel micropipette assembly was introduced by D. R. Curtis (Curtis 1964). Neuronal spikes can be recorded through a glass micropipette if filled with a suitable electrolyte solution such as sodium chloride. However, electrolyte-filled micropipettes in a multibarrel assembly are electrically very "noisy". The solid-conductor microelectrodes such as the tungsten or carbon fiber electrodes, in contrast, exhibit significantly less noise in extracellular recordings. For this reason, many attempts have been made to combine a tungsten electrode with a set of iontophoresis barrels. The usual technique has been to glue the two types of electrodes together (piggyback configuration; Krnjevic 1971) or to insert a presharpened tungsten wire into one of the barrels (metal-in-glass configuration; Kasser and Cheney 1983; Hellier et al. 1990; Li et al. 1990; Godwin 1993; Haidarliu et al. 1995). Either method involves a technically very difficult and time-consuming procedure.

The carbon fiber-containing recording/microiontophoresis combination electrodes are considerably easier and cheaper to make than tungsten electrode-containing multibarrel assemblies. The single most difficult task in making carbon fiber microelectrodes is to attain the correct length and shape of the carbon tip protruding from the glass micropipettes. In our experience, cutting with fine scissors proved very tedious and the rate of successfully finished electrodes was low. Etching by means of an alternating current in a drop of dilute chromic acid or physiological saline proved to be a more reliable method and produced better carbon tips. However, the tip of a finished microelectrode must be kept in distilled water overnight in order to remove the acid or salt contamination from the iontophoresis barrels. As a rule, once the iontophoresis barrels have been filled with any aqueous solution, the tip must never be allowed to dry out. Otherwise, the microcrystals formed damage the fine glass configuration in the tip and the electrode becomes useless due to the production of excessive electrical noise. To circumvent these problems, we introduced and improved the spark etching method to achieve sharp-pointed conical carbon tips protruding 15-20 µm from the glass assembly. Since no solutions are involved, this dry method means that such microelectrodes may be stored in a dust-free place for many weeks without loss of their good recording qualities. In consequence of the inner glass filaments, the iontophoresis barrels are simple to fill, and the sharp tip penetrates tissues with ease. These carbon tips may additionally be suitable for electrochemical measurements and for the development of enzyme- or antibody-based microbiosensors (Kawagoe et al. 1991a; Garguilo and Michael 1996; Netchiporouk et al. 1996; Darbon et al. 1998).

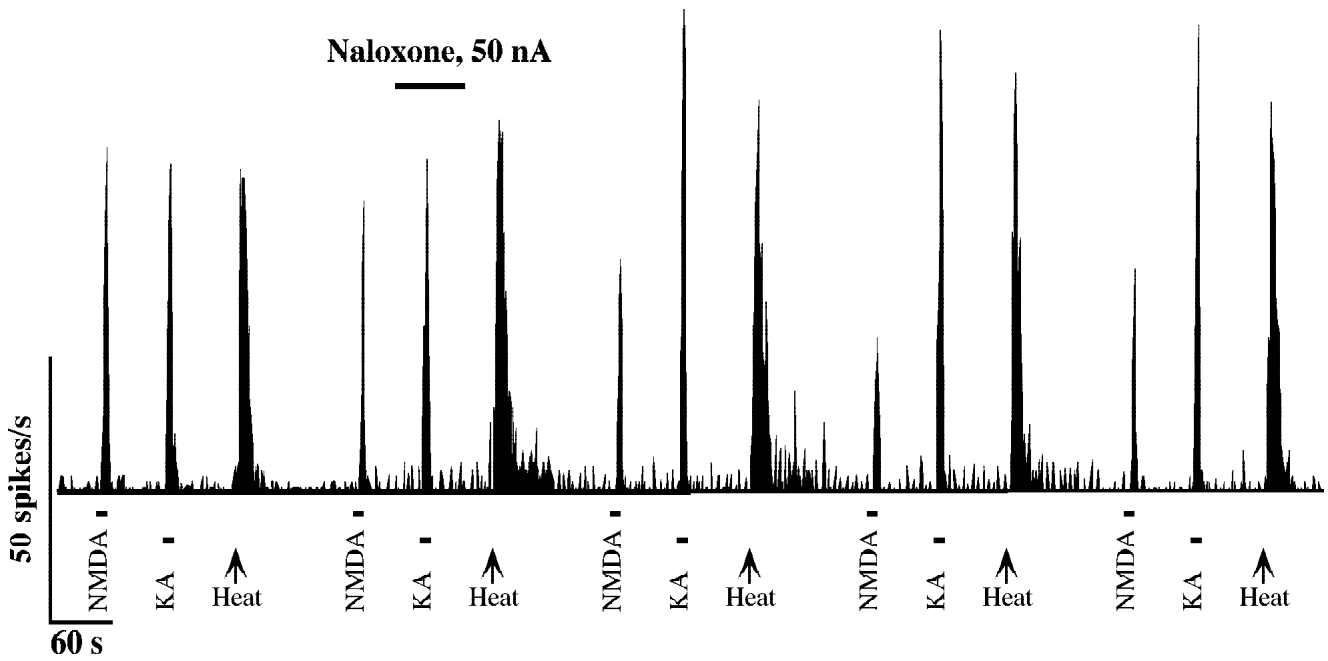

Figure 6. Ratemeter recordings showing the effects of iontophoretically applied naloxone on a wide dynamic range (WDR) dorsal horn neuron excited by iontophoresed NMDA (ejected at -52 nA for 5 s) and KA (-15 nA, 5s) and noxious heat stimulation of the cutaneous receptive field. Drugs were ejected as indicated by the horizontal bars. Note the facilitating effect of naloxone on the KA- and heat-evoked responses. In contrast, the responses to NMDA were inhibited in the presence of naloxone.

Acknowledgments

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