Method for the construction and use of carbon fiber multibarrel electrodes for deep brain recordings in the alert animal

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ABSTRACT

Microiontophoresis of neuroactive substances during single unit recording in awake behaving animals can significantly advance our understanding of neural circuit function. Here, we present a detailed description of a method for constructing carbon fiber multibarrel electrodes suitable for delivering drugs while simultaneously recording single unit activity from deep structures, including brainstem nuclei and the cerebellum, in the awake behaving primate. We provide data that should aid in minimizing barrel resistance and the time required to fill long, thin multibarrel electrodes with solutions. We also show successful single unit recording from a variety of areas in the awake squirrel monkey central nervous system, including the vestibular nuclei, Interstitial Nucleus of Cajal, and the cerebellum. Our descriptions and data should be useful for investigators wishing to perform single unit recordings during microiontophoresis of neuroactive substances, particularly in deep structures of animals with chronically implanted recording chambers.

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1. Introduction

Simultaneous single unit recording and microiontophoresis of neuroactive substances in alert behaving animals can significantly advance our understanding of how neural circuits utilize neurochemicals to control the firing behavior of their constituent neurons, and thus produce behavior. Despite the wide use of this technique in anesthetized preparations (Millar and Williams, 1989; Williams and Millar, 1990; Cudeiro et al., 1997), few labs that work with alert behaving animals employ simultaneous single unit recording and microiontophoresis in their investigations (Alloway and Burton, 1991; Herrero et al., 2008). One reason for the underutilization of such a powerful technique may be the perceived difficulty of producing multibarrelled glass electrodes that are suitable for recording and iontophoretically delivering drugs in chronically implanted animals on a daily basis.

The potential of microiontophoresis has been recognized for decades by investigators working with anesthetized animals (Millar and Williams, 1989; Williams and Millar, 1990; Rivadulla et al., 2003), and the majority of methods papers to date deal with optimizing the technique for these preparations (Armstrong-James and Millar, 1979; Armstrong-James et al., 1980; Anderson and Cushman, 1981; Millar and Williams, 1988; Fu and Lorden, 1996; Kuras and Gutmaniene, 2000). The technique has been around for over 50 years (Curtis and Eccles, 1958), but Armstrong-James and Millar (1979) made a drastic improvement in the quality and reliability of the single unit recordings that can be achieved by introducing the use of a carbon fiber as the recording electrode. This design has been further improved over the years by introducing new methods of etching the carbon fiber tip (Armstrong-James et al., 1980; Kuras and Gutmaniene, 2000) and speeding up the manufacturing process (Anderson and Cushman, 1981; Fu and Lorden, 1996; Millar and Pelling, 2001). However, few papers have dealt with the unique challenges faced by investigators who wish to apply the technique to record from chronically implanted awake behaving animals (Alloway and Burton, 1991), namely penetration of the dura and scar tissue, sterilization of the electrodes, and access to deep structures.

We have spent several years perfecting the manufacture and use of carbon fiber multibarrel electrodes in alert behaving squirrel monkeys and offer here a detailed description of a complete system for daily recording and microiontophoresis in deep structures, including brainstem and cerebellar sites. These electrodes can be produced in under 15 min and provide excellent signal-to-noise characteristics. We describe their manufacture, providing several suggestions to simplify the process; present results from a parametric study of the optimal characteristics for a variety of electrode configurations; and show their suitability for recording and

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simultaneous iontophoretic drug delivery in several deep structures of the alert behaving primate.

2. Material and methods

2.1. General supplies

Multibarrel electrodes are constructed from preassembled borosilicate multi-capillary glass tubing and carbon fibers. Pre-assembled three barrel glass was purchased from World Precision Instrument (Sarasota, FL, USA), four barrel glass from A-M Systems (Carlsborg, WA, USA), and seven barrel glass from FHC (Bowdoin, ME, USA). Bundles of carbon fibers consisting of individual 5 or 7 μm fibers were obtained from TORAY carbon fiber USA. Stainless steel and polyimide tubing were purchased from Small Parts Inc., and were used to make guide tubes and to help separate individual carbon fibers, respectively.

2.2. Animal preparation and recording setup

Three squirrel monkeys (monkey 062, monkey 066, and monkey 408) 4–10 years of age were used for these experiments. We used standard surgical procedures performed under Isoflurane anesthesia and aseptic conditions in a fully equipped surgical suite (Blazquez et al., 2003, 2007). In a first surgical procedure we implanted a stainless steel post for head fixation and an eye coil to monitor the eye position. In a second surgery we implanted a chamber for neuronal recordings. Surgeries were separated by a minimum period of 3 weeks to allow for animal recovery. Surgical methods and experimental protocols were approved by the Washington University Committee on Animal Care, and were performed in accordance with the National Institute of Health guidelines.

Animals were comfortably seated in a primate chair during experiments. Our recording setup consists of a custom-made AC coupled differential amplifier, a hydraulic microdrive, a Neurophore BH-2 iontophoretic pump system (Medical Systems Corp.), and a search coil eye movement detector system (Neuro Data Instrument Corporation). A Power 1401 (Cambridge Electronic Design) connected to a PC computer and running Spike2 software (Cambridge Electronic Design) was employed for data acquisition (neuronal and behavioral) and stimulus presentation. In addition we use several servo-controlled motors in our experiments, the details of which are not important for this manuscript.

2.3. Manufacture of the carbon fiber multibarrel electrode

Others have already reported that the use of a carbon fiber as the recording electrode considerably improves the recording quality compared with a saline-filled barrel alone (Armstrong-James and Millar, 1979; Fox et al., 1980). The carbon fiber can be placed either inside one of the barrels or in the center space between barrels. Below we describe methods we have developed to simplify the manufacture of multibarrel electrodes containing a carbon fiber recording filament.

2.3.1. Separation of individual carbon fiber filaments

A commercial bundle of carbon fibers contains upwards of 10,000 individual carbon fiber filaments, which can be as small as 5–7 μm each. The small size and fragility of each filament make the process of separating carbon fiber bundles into individual filaments tedious. Others have suggested the use of specially modified forceps to remove individual filaments from the bundle (Armstrong-James and Millar, 1979; Fu and Lorden, 1996), however we find it easier to separate fibers by placing the bundle of carbon fibers on a sheet of glossy photographic inkjet paper (e.g. Kodak), on which we extract individual filaments with the help of a 28 gauge polyimide tube

under a dissection microscope (6× magnification); polyimide tubes are flexible and do not break the individual filaments upon contact. The carbon fibers stick lightly to the glossy paper, which prevents them from being blown away from air drafts. Once separated, single carbon fibers can be left on the glossy paper where they remain until needed.

2.3.2. Inserting the carbon fiber into a glass barrel

The difficulty of inserting a 5–7 μm carbon fiber filament into one of the glass barrels can be one of the biggest impediments to the successful manufacture of multibarrel electrodes. We have improved current published methods to make this step less time consuming and onerous. The first method we present is similar to one employed by Fu and Lorden (1996) in which a narrow tungsten wire (100 μm, Small Parts Inc.) a couple of centimeters or more longer than the borosilicate glass is inserted in one of the glass barrels. Under the dissection microscope, a single carbon fiber is then glued to one end of the wire using cyanoacrylate glue. After the glue dries, the wire is slid through the glass barrel such that it carries the carbon fiber through the full extent of the glass barrel to the other end. The second method can be performed much more rapidly than the first, requiring only a length of polyimide tube (28 gauge) a couple of centimeters longer than the borosilicate glass. We insert 1–2 mm of the carbon fiber into one of the glass barrels either by hand or by lifting the fiber with the polyimide tube and sliding the glass barrel over the fiber. Under the dissecting microscope, the polyimide tube is then introduced into the same glass barrel and pushed through until it exits the other end. The friction and electrostatic forces between the carbon fiber and the polyimide tube ensure that the carbon fiber is carried to the other end of the glass barrel with the polyimide tube. This method requires some practice but it is cleaner (no glue) and faster, and is our method of choice. Once it is loaded into the glass barrel, we cut the carbon fiber several centimeters from the end of the glass so that only a few centimeters protrude on either end. Finally, we place a mark on the barrel containing the carbon fiber to keep track of its location as the fiber is difficult to see with the naked eye.

2.3.3. Pulling the multibarrel electrode

We use a horizontal multi-pipette puller (PMP-107L, Micro Data Instrument, S. Plainfield, NJ, USA) because this puller allows for the construction of long, thin tips, but any multistage glass puller that can accommodate multibarrel glass should work as well. While the glass is being pulled and separated into two segments it is critical that the carbon fiber remains static with respect to the glass segment that will ultimately form the multibarrel electrodes, otherwise the glass will not form a tight seal around the carbon fiber at its tip. The best approach we have found to achieve this with high success is simply to stabilize the carbon fiber protruding from the glass by holding it between our thumb and forefinger during the pulling stages. Alternatively, one may place a small drop of cyanoacrylic glue at the end of the barrel containing the carbon fiber, taking care not to completely obstruct the barrel. Once the glass electrode is pulled, the carbon fiber should be attached to the half of the glass that forms the electrode. Finally the carbon fiber is cut with small scissors at both ends leaving only about 5 mm of extra carbon fiber protruding from the tip of the multibarrel electrode, and no extra carbon fiber protruding from the back of the multibarrel electrode. The carbon fiber will be a uniform diameter along its entire length because it does not taper when heated and pulled. Once pulled these electrodes can be stored for up to 1 week in a dry container. We do not recommend storing electrodes beyond this 1-week period, as we have found that longer storage times (we have tried up to one month) lessen the chances of successfully filling the barrels with solution.

The manufacturing process up to this point takes about 10 min. The remaining part of the manufacturing process is performed just before the experiment.

2.3.4. Filling the electrodes with drugs

Each barrel is back-filled using a 34 gauge microfil syringe (MF34G, World Precision Instruments). Because our electrodes are unusually long and thin compared with multibarrel electrodes used in vitro and in more superficial structures in vivo they need long times to fill (see Fig. 2D). Electrodes must be inspected under the microscope to ensure adequate filling, as electrodes with air bubbles are inadequate for delivering drugs using DC current and must be discarded (Lalley, 1999). In our experience, the chance of encountering bubbles in the electrodes increases with longer tips size. We also found that the type of solution matters. Bubbles are rare in solutions containing substances that easily dissolve (e.g. GABA, gabazine in 165 mM NaCl), while they are frequently found in barrels with solutions containing substances that are hard to dissolve (e.g. bicuculline in 165 mM NaCl). Filters with small pore sizes (ISO-Disc Filters 0.2 μm, Supelco) are necessary when using the latter type of solution.

Before etching the tip of the carbon fiber, all the barrels are filled with the solutions of choice. This is important because etching the carbon fiber tip can contaminate the neighboring glass tips with carbon residue, which could prevent them from filling.

2.3.5. Tip forming

Electrodes in which the carbon fiber has been carefully trimmed at the tip can be used to record multiunit activity and large or sparsely packed neurons in which small tip sizes are not necessary, but we prefer to etch the carbon fiber tip to reduce its surface area (Fig. 1A). To etch the tip of the carbon fibers we use a setup similar to that employed by other investigators, consisting of a standard AC transformer (9 V, 60 Hz) that passes current between the tip of the carbon fiber and a salt solution (Fu and Lorden, 1996). One of the leads of the AC source is connected to a thin metal wire, such as copper or tungsten, which has been bent to form a small hook at its end. A small drop of salt water (e.g. 165 mM NaCl) is placed in this hook. The second lead of the AC source is connected to the carbon fiber. Other investigators have used silver paint or other types of conductive glue to attach the carbon fiber to a connector (Fu and Lorden, 1996; Kuras and Gutmaniene, 2000; Millar and Pelling, 2001), which is then attached to the second lead of the transformer. This procedure can be time consuming, thus complicating the fabrication process. Instead we use a saline solution (e.g. NaCl, 165 mM) as the interface between the carbon fiber and the transformer. The barrel containing the carbon fiber is filled with salt solution, and then a silver wire attached to the second lead of the AC source is introduced into the barrel. During recording the same saline solution will serve as the interface between the amplifier headstage and the carbon fiber. Eliminating the use of silver paint simplifies the manufacture of our multibarrel glass electrodes without compromising the quality of the recording (see Figs. 3–5).

The optimal length of carbon fiber protruding from the tip depends on the target of recording; short tips (about 5 μm) are preferable to record activity in areas with densely packed neurons (e.g. Purkinje cell layer in the cerebellar cortex), while long tips (15–20 μm) are preferable in areas with large and sparse neurons (e.g., big neurons in the deep cerebellar nuclei or reticular formation).

2.3.6. Guide tube system

We use a custom-built guide tube system to protect our electrodes as they are introduced into the brain. These guide tubes are built using stainless steel hypodermic tubes of increasing size from 25G to 7G. The smaller gauge (23G or 25G) penetrates the brain
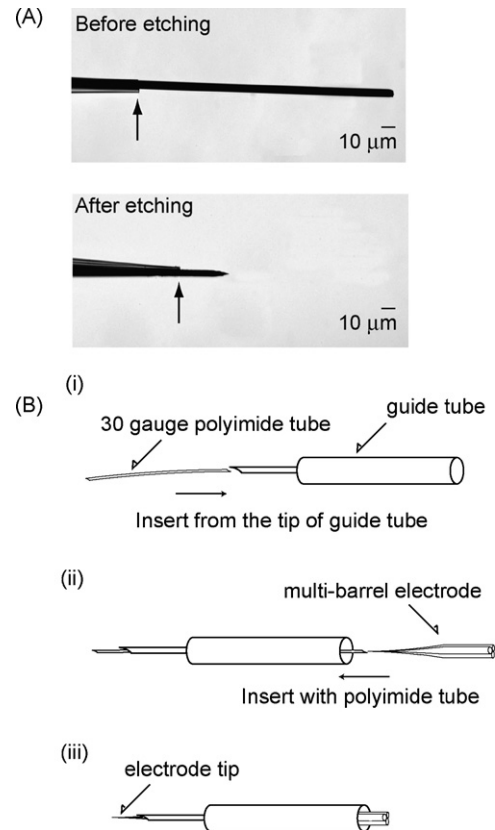

Fig. 1. Etching carbon fiber (A) and loading multibarrel electrode into guide tube (B). (A) Photomicrographs of multibarrel electrode tip before (top) and after (bottom) etching the carbon fiber. Arrows indicate edge of glass. (B) Schematic representation of three step process to load multibarrel electrode into guide tube: (i) insert polyimide tube into front of guide tube; (ii) under a dissection microscope, gently insert tip of electrode into polyimide tube; (iii) push electrode and polyimide forward. The polyimide tube will protect electrode tip as it passes through guide tube.

while the larger gauge (which is hollow) holds the body of the multibarrel electrode. Additionally, the large gauge easily fits our X–Y positioner system (Trent Wells). We prefer this compound guide tube system over a simpler single diameter guide tube because it stabilizes the multibarrel electrode and protects the glass from accidental breakage due to shearing forces.

2.3.7. Sterilization method

Because our electrodes are used for chronic recordings it is important to prevent infection by sterilizing any element that will come into contact with the brain. We use a two-step sterilization process. In a first step electrodes are placed in a UV sterilizer for 1–2 h. UV sterilization is typically used for surface sterilization but in our case, because we use glass, both glass and solution are sterilized. In the second part of the sterilization process we dip the tip of the multibarrel electrode in 70% alcohol for a few seconds. Our sterilization process does not seem to alter the effectiveness of the drugs (bicuculline, gabazine, GABA, glutamate, DL homocysteic acid, and baclofen).

Guide tubes, X–Y positioner, and polyimide tubes are sterilized in a 70% alcohol solution or an autoclave.

2.3.8. Loading multibarrel electrode into guide tube

Multibarrel electrodes are normally used in experiments that do not require the use of guide tube systems, such as anesthetized preparations where the dura mater is removed before the experiment, or in slice recordings. However, during chronic recordings guide tubes are necessary to protect the tip of the electrodes from

the hard layers of scar tissue and dura mater. For chronic recordings it is preferable to leave the dura mater, and sometimes also the scar tissue above, intact to reduce the chances of infection. Regular metal microelectrodes can be placed inside the guide tube by passing the back of the electrode through the front of the guide tube in order to protect the delicate electrode tip; however this is not possible with multibarrel electrodes. We suggest a simple and highly successful method to insert the multibarrel electrode inside the guide tube without breaking its tip (Fig. 1B). This method consists of first inserting into the guide tube a 30 gauge polyimide tube with one end cut at an angle (Fig. 1Bi). The polyimide tube protrudes from both ends of the guide tube. Under a dissection microscope we carefully place the tip of the multibarrel electrode through the angled end of the polyimide tube, and then carefully advance the guide tube towards the multibarrel electrode (Fig. 1Bii). The polyimide protects the tip of the electrode as it passes through the guide tube (Fig. 1Biii).

2.3.9. Other suggestions to improve the success of recording

Although the step-by-step process of building glass multibarrel electrodes with carbon fiber described above should be sufficient to successfully build these electrodes, there are tricks that, if implemented correctly, significantly increase the success of recordings.

1. Dip the tip of the multibarrel electrode in sterile distilled water for 5 min before putting it inside the brain. This dissolves the salt crystals that form at the tip of the electrode and removes any alcohol residue.
2. One of the biggest problems we have encountered in our recordings is electrical shorting between barrels. To reduce the chance of this occurring, gently break the back end of each barrel with a pair of sharp forceps (after the barrels have been filled with solution). This breaks the glass filament inside the barrels and prevents the formation of an electrical bridge between barrels through the salt solution. We usually do this just before recording, after the electrode has been loaded into the guide tube and microdrive system. In addition, all electrical connections between the recording barrel and amplifier, and the injection barrels and iontophoretic pump should use insulated wire to prevent shorting between barrels.
3. Do not store the fabricated unfilled electrodes for more than a week. Electrodes that have been stored for long period of time (a week or more) tend to form more bubbles, possibly due to the accumulation of dust.
4. Always examine the electrodes under the microscope before introducing them into the brain, as the tip sometimes gets accidentally broken while handling the electrode in the final stages.
5. Before introducing the guide tube into the brain, clear a path for it by first puncturing the scar tissue and dura mater with a sharpened stainless steel tube.
6. Always try to minimize external sources of electromagnetic noise, as these electrodes can be more susceptible to interference than regular metal microelectrodes. For instance, we use an eye coil system to measure eye movements and have found that we need to optimize the current intensity to the field coils to give good spatial resolution without introducing too much recording noise. The field coils are usually the major source of noise during our recordings. Decreasing the intensity of the field coils can completely eliminate this noise. In our recordings the intensity of the field coils is reduced to about 30% of its maximum. The resulting eye position spatial resolution is 0.09 degrees, above the minimum required for our experiments.
7. Once a barrel becomes blocked or otherwise stops passing current, it is unlikely that the barrel can be recovered and it is best to replace the electrode.

3. Results

3.1. General characteristics of multibarrel electrodes

We performed a series of measurements on a variety of electrode configurations to determine the "best" multibarrel electrode characteristics for achieving successful recording and iontophoretic injection. The properties we were concerned with were (1) the DC resistance of individual barrels, which gives an indication of both how susceptible the barrel is to clogging during an experiment and how much current can be delivered by the iontophoretic device; and (2) the time required to completely fill the barrels with solution, which determines the convenience and feasibility of preparing the electrodes for an experiment. We varied the concentration of sodium chloride in the filling solution (salinity) and the length of the electrode taper and found that both factors are key determinants affecting the resistance of the barrels and the ability to fill them in a reasonable period of time.

The electrodes used in the following section were all pulled so that they fit inside a 25 gauge guide tube (25G extra thin wall, Small Parts) for at least half of their length. The length of the taper was defined as the distance from the shoulder of the electrode where its shank begins to taper until its tip. Visual inspection under a light microscope revealed that the tip diameter of our multibarrel electrodes was 2–3 μm, with no difference among the three, four and seven barrel electrode configurations. The measurements in the following section were repeated on 10 barrels for each condition. All statistical tests for a significant effect of salt concentration or taper length on barrel resistance or fill time were performed using the Spearman Rank Coefficient test because a Bartlett test revealed that the different groups did not meet the equal variance assumption required by ANOVA and many other statistical tests.

We filled multibarrel electrodes with sodium chloride solutions of several molarities. In those barrels that were free of bubbles after a reasonable waiting period (<4 h) we measured the resistance
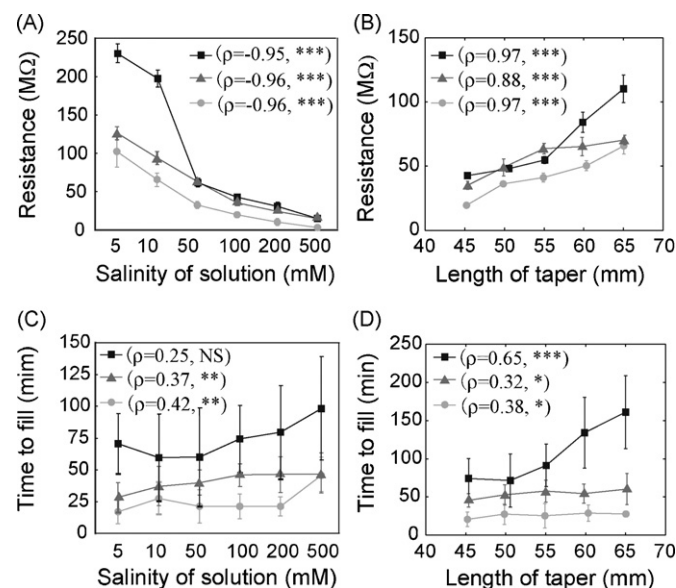
Fig. 2. Measurements of barrel resistance (A and B) and barrel fill time (C and D) for three (circle), four (triangle), and seven (rectangle) barrel electrodes. (A and B) Barrel resistance as a function of sodium chloride concentration (salinity; A) or taper length (B) for all three electrode configurations. (C and D) Fill time as a function of salinity (A) or taper length (B). (A and C) All electrodes had 45 mm taper length. (B and D) All barrels were filled with 100 mM NaCl. All values are mean ± 1 SD for 10 barrels. In each panel, ρ and asterisk, respectively, denote Spearman's rank correlation coefficient and level of significance of correlation in the Spearman's rank test: *(p < 0.05), **(p < 0.01), ***(p < 0.001) and NS (no significance).

with a Neurophore BH-2 (Medical system corporation, Great Neck, NY, USA) after placing the tip of the electrode in a beaker filled with 0.9% sodium chloride irrigation solution (Baxter Healthcare Corp., Deerfield, IL, USA) to approximate the extracellular resistance. Within the range of molarities that we tested, we found that the DC resistance of the barrels decreased as the molarity of the saline solution was increased ($p < 0.001$ for all barrel configurations), reaching an asymptote around 150 mM NaCl (Fig. 2A). This reduction in resistance comes at the cost of a decreased yield, as more of the electrodes that were filled with a high salt concentration had to be rejected due to incomplete filling or broken tips from salt crystallization. Thus saline concentrations above 150 mM offer little advantage in terms of reducing the barrel resistance.

Next, we investigated the dependence of barrel DC resistance on the length of the electrode taper. We constructed electrodes with three, four, and seven barrel configurations, each ranging in taper length from 45 to 65 mm. We filled all electrodes with 100 mM sodium chloride solution and measured their DC resistance using the Neurophore BH-2 and a 0.9% saline external solution. Fig. 2B presents the measured resistances for the multibarrel electrodes. Within each barrel configuration, the barrel resistance monotoni-

cally increased as a function of taper length ($p < 0.001$ for all barrel configurations; see Fig. 2 for individual correlation coefficients and p-values). In agreement with Fig. 2A, the three barrel electrodes had the lowest overall resistance and the four and seven barrel electrodes had fairly comparable resistance, at least for the shorter taper lengths.

We have found that barrels with a resistance above 50 MΩ often become clogged during the course of an experiment, forcing us to replace the electrode. In order to avoid this, we generally strive to use electrodes with barrel resistance below this 50 MΩ threshold. Based on the measurements reported in Fig. 2A and B, this can be achieved with NaCl concentrations above 50 mM for three barrels and 100 mM for four and seven barrels. This resistance threshold also limits the maximum electrode taper length that can be used. That is, taper lengths of up to 60 mm can be used with the three barrel configuration, while the four and seven barrel configurations are limited to 50 mm tapers.

As stated above, the reduction of barrel resistance created by higher salt concentrations must be weighed against the increased time required to fill the electrodes and the increased likelihood of bubble formation or tip breakage with these solutions. Fig. 2C shows
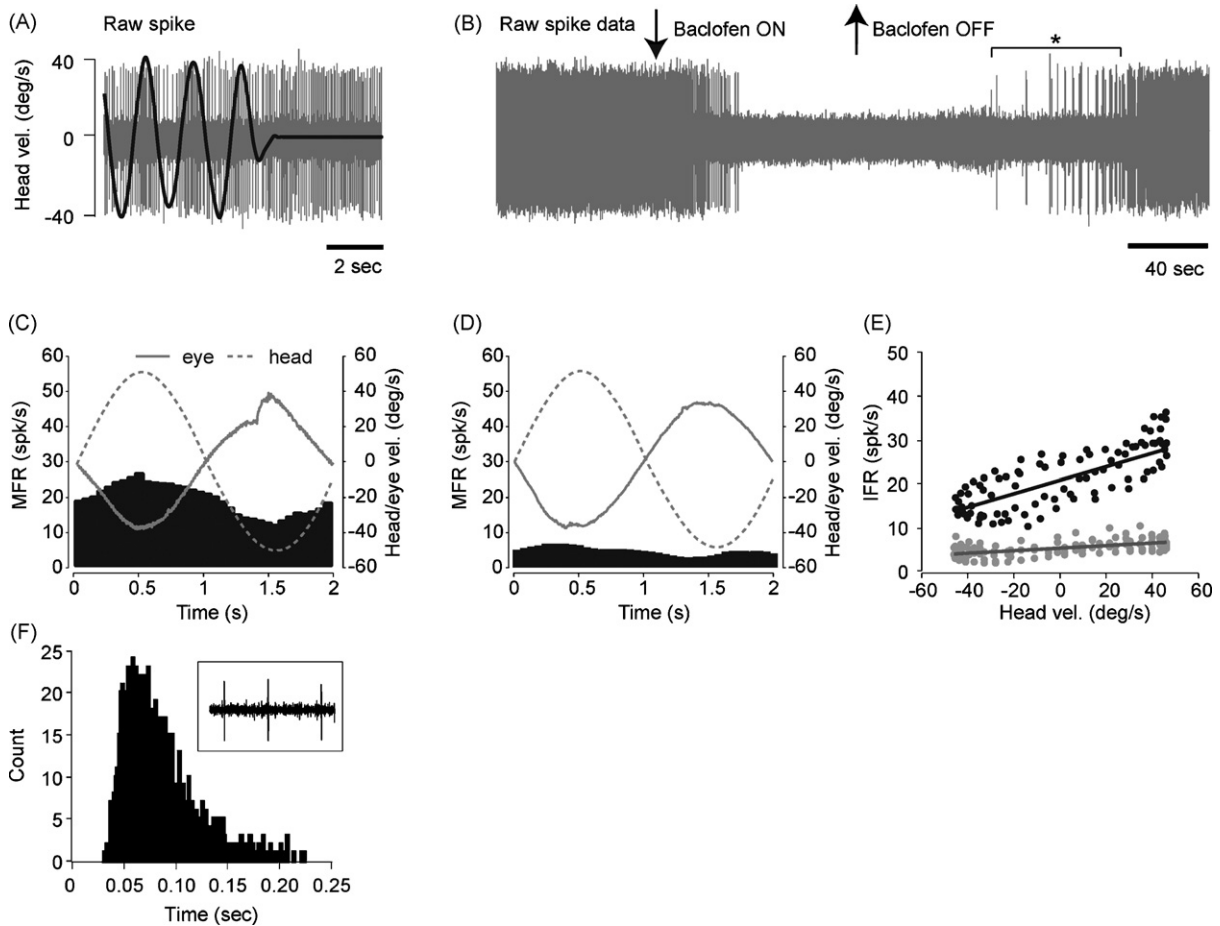

Fig. 3. Vestibular-only (VO) neuron in superior vestibular nucleus (SVN) recorded before and during injection of the GABA-B agonist baclofen (50 mM, in 165 mM NaCl, pH 3.5) from one barrel of three barrel electrode (one barrel has the carbon fiber). (A) Extracellular recording of the VO neuron (gray) with sinusoidal vestibular stimulus overlaid (black) during retention of baclofen in the barrel (-30 nA retention current). The neuron firing rate is modulated in phase with the vestibular stimulation (head velocity; C). (B) Extracellular recording for a longer period of time showing time course of neuron firing rate decrease during the iontophoretic injection of baclofen (40 nA injection current). After the baclofen injection was stopped by applying a retention current, but before the neuron firing rate had recovered to its preinjection level, the vestibular stimulation was applied again (section indicated with asterisk; stimulus not shown for clarity), causing modulation of the neuron with a lower DC firing rate. Mean stimulus velocity, eye velocity and firing rate during vestibular stimulation before and 1–2 min after baclofen application are shown in (C) and (D), respectively. Data shown in (D) correspond to a portion of the recording were the neuron was still recovering from injection of baclophen (see asterisk in (B)). (C and D) Black histogram is mean firing rate of neuron, gray dashed line is mean head velocity, and gray solid line is mean eye velocity. Bin size for histograms is 50 ms. (E) Instantaneous firing rate (IFR) and head velocity pre (black dots) and post injection (gray dots). Both black and gray solid lines are regression lines. Sensitivity for head velocity and baseline firing rate are 0.15 spk/s/deg/s and 20.7 spk/s before baclofen injection and 0.03 spk/s/deg/s and 5.2 spk/s after baclofen injection. (F) Interspike histogram showing that the neuron was well isolated (the time between two consecutive spikes was always above 25 ms). Also in the inset we show a magnified portion of the raw data (100 ms).

the time required to fill all barrels of the three, four, and seven barrel electrode configurations using a variety of sodium chloride concentrations ranging over two orders of magnitude (5–500 mM NaCl). Within the range of concentrations we used, there was a small but significant dependence of fill time on saline concentration for the three and four barrels ($p < 0.01$), but not for the seven barrel configuration ($p > 0.05$). Overall, the three barrel elec-
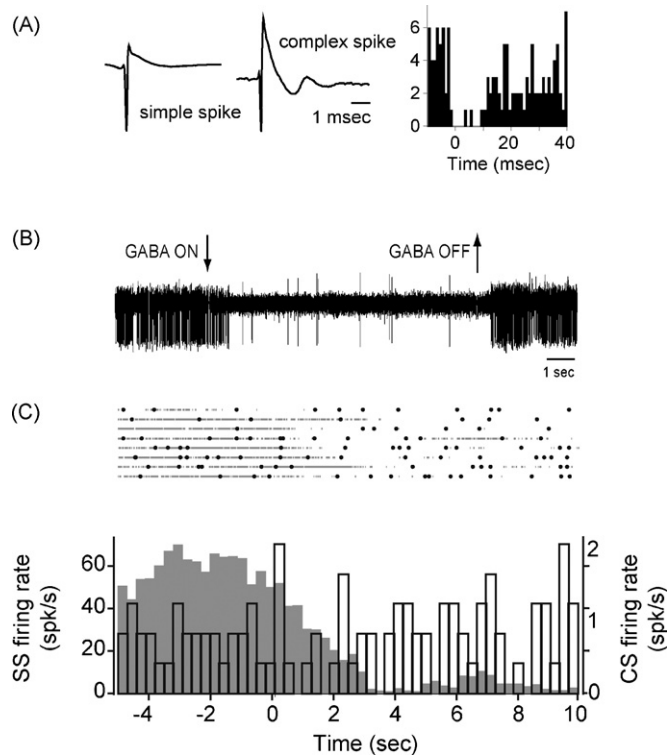
Fig. 4. Y-Group flocculus target neuron (FTN) recorded before and during injection of the excitatory amino acid DL-homocysteic acid (DLH; 100 mM, in 165 mM NaCl, pH 3.5). (A) Time course of DLH injection is shown. Top, instantaneous firing rate (IFR) of neuron; middle, vestibular stimulation; bottom, optokinetic stimulation (OKS). First block of stimulation is optokinetic only (visual following); second block is vestibular ocular reflex (VOR) cancellation induced by paired in phase rotation of vestibular table and optokinetic drum. (B and C) Mean firing rate, stimulus velocity, and eye velocity before (B) and during (C) iontophoretic DLH application. Format is same as Fig. 3, with the addition of average OKS trace (dotted line). Bin size for histograms is 50 ms. (D) IFR plotted versus head velocity during VOR cancellation (left) or eye velocity during visual following (right). The format is the same as Fig. 3. In the VOR cancellation, sensitivity for head velocity and baseline firing rate are 0.28 spk/s/deg/s and 64.4 spk/s before DLH injection and 0.47 spk/s/deg/s and 138.2 spk/s during DLH injection. In the visual following, sensitivity for eye velocity and baseline firing rate are 0.18 spk/s/deg/s and 50.6 spk/s before DLH injection and 0.24 spk/s/deg/s and 135.2 spk/s during DLH injection.

Fig. 5. Recording of cerebellar Purkinje cell before, during, and after iontophoretic GABA injection (30 nA, 500 mM, in 165 mM NaCl, pH 3.5). (A) An example of simple spike (left), complex spike (middle) and simple spike interval histogram triggered by complex spike (right). The complex spikes show a characteristic longer "tail" than the simple spikes. (B) Extracellular recording of simple and complex spikes from Purkinje cell, including time course of GABA injection. When GABA was retained in the barrel, the Purkinje cell had a high spontaneous discharge. Within a couple of 100 ms of applying a 30 nA current to inject GABA, the simple spikes were almost completely shut down, leaving only complex spikes. When the GABA was again retained in the barrel, the neuron quickly recovered and began producing simple spike discharges. (C) Raster plot of simple spikes (gray lines) and complex spikes (black dots) and peristimulus time histogram (PSTH) of them (gray, simple spike; open black, complex spike). The peri-stimulus time histogram was constructed from 8 repetitions of 10 s injection of GABA. Bin size for the histogram is 300 ms. Time zero in the PSTH is the onset of GABA injection.

trodes filled the fastest and the seven barrel electrodes filled the slowest.

We next examined whether the time required to fill the barrels is influenced by the electrode taper length. Fig. 2D summarizes the time required to fill electrodes constructed with different taper lengths using a 100 mM saline solution. Barrel length had a slight but significant effect on the time required to fill the barrels when using the three or four barrel configurations ($p < 0.05$), and a larger effect with the seven barrel configuration ($p < 0.001$).

Thus, once an electrode configuration is chosen, there is a wide range of salt concentrations and taper lengths that can be used without any major difficulties filling the barrels. With three and four barrel configurations the limiting factor will likely be the consideration of barrel resistance, whereas fill time only really becomes an issue with the seven barrel configuration. Note, however, that as the number of barrels increases, the number of barrels that must be successfully filled in order to use the electrode also increases. For this reason, we usually choose the three or four barrel over the seven barrel configuration.

3.2. Example recordings and iontophoretic injection in different brain areas

To test the suitability of our electrodes for use in a variety of deep brain structures we performed recordings and simultaneous
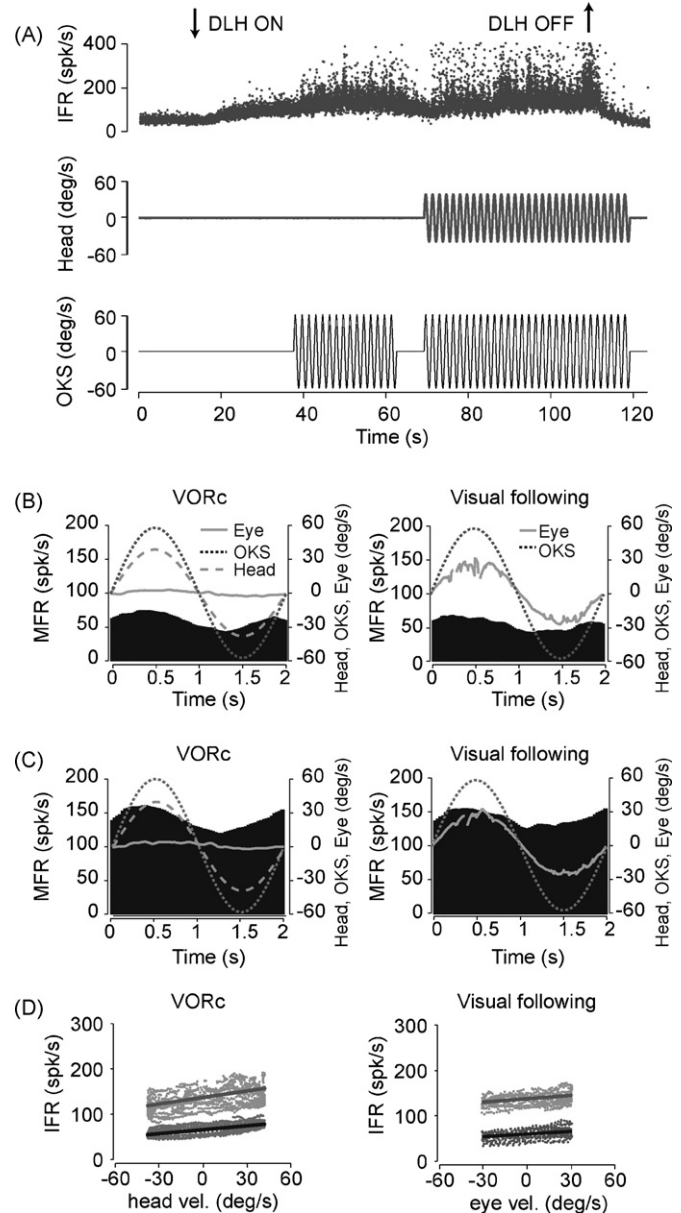
iontophoretic injections from several locations in the central nervous system of the squirrel monkey. For this purpose, we used three or four barrel electrodes up to 50 mm in length because we have found that these are among the easiest to work with for the reasons presented above. The barrel that contains the carbon fiber was filled with saline and used for recording (see Section 2). A second barrel filled with 165 mM sodium chloride solution was used for current compensation. The remaining barrels were filled with solutions containing the drugs of choice (baclofen/DL homocysteic acid/GABA/bicuculline).

Fig. 3 presents an example recording from a superior vestibular nucleus neuron that responded only during vestibular stimulation (whole body rotation; Fig. 3A and B). This recording has a good signal-to-noise ratio and the spikes can be clearly separated from the background activity. One barrel of the three barrel electrode contained the GABA-B agonist baclofen, which was retained in the barrel with a −30 nA DC current. When a 40 nA current was injected to deliver the drug in the vicinity of the neuron, the neuron responded within about 30 s by gradually decreasing its firing rate until it was almost completely shutdown, indicating the presence of functional GABA-B receptors within the area reached by the drug (Fig. 3C) (Herz et al., 1969). The neuron still responded during head rotations, but with reduced amplitude compared with the control (compare Fig. 3B and D).

Fig. 4 presents a putative flocculus target neuron (FTN) recorded in the Y-Group nucleus. This neuron responded during both eye movements evoked by optokinetic stimulation (visual following) and vestibular stimulation during vestibular ocular reflex (VOR) suppression. When the excitatory amino acid DL-homocysteic acid was injected, the neuron quickly responded by increasing its mean firing rate (Fig. 4A). This increase in firing rate did not change the gain of the neuron during optokinetic or vestibular stimulation (Fig. 4B and C).

Our multibarrel electrodes are also capable of crossing the layer of dura mater covering the cerebellum (tentorium). Fig. 5 shows a Purkinje cell recorded in the flocculus with a three barrel electrode in which one barrel contains GABA. The recording quality is similar to that generally achieved for flocculus Purkinje cells with a tungsten electrode. When GABA was injected with a 30 nA current, the neuron immediately responded by substantially reducing its firing rate. The neuron quickly recovered when the GABA injection was stopped by applying a −30 nA retention current, indicating that GABA is readily removed from the synaptic space.

The real power of multibarrel electrodes is apparent when we load two or more different drugs into the barrels and examine their respective contributions to the neuron firing behavior. Fig. 6 presents a neuron recorded in the Interstitial Nucleus of Cajal (INC). We used a four barrel electrode, with one barrel containing GABA
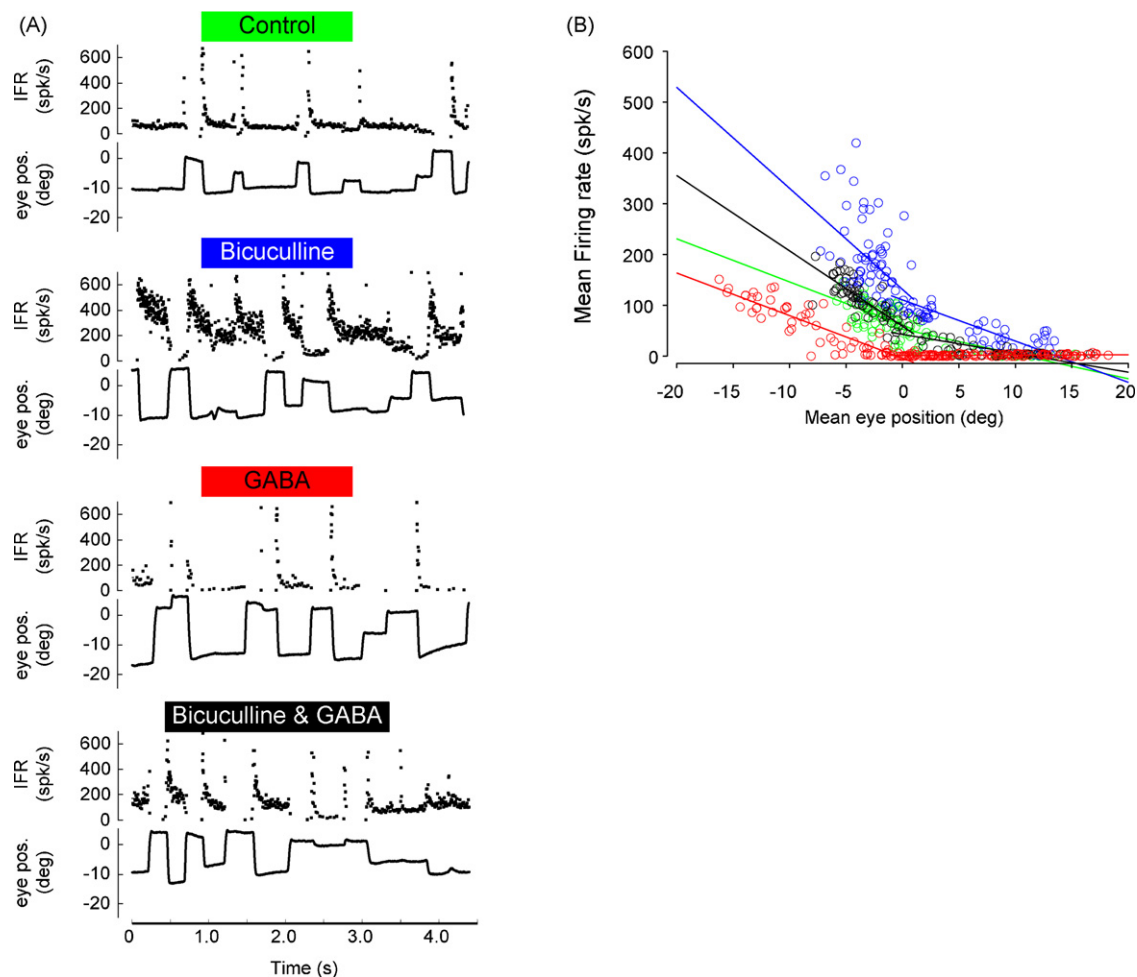

Fig. 6. Recording of neuron in the Interstitial Nucleus of Cajal (INC) with four barrel electrode containing both GABA (500 mM, in 165 mM NaCl, pH 3.5) and the GABA-A antagonist bicuculline (20 mM, in 165 mM NaCl, pH 3.5). (A) Example neuron responses during spontaneous eye movements under four conditions: top panel, retention of both drugs; second panel, injection of bicuculline only; third panel, injection of GABA only; bottom panel, injection of both bicuculline and GABA. In each panel, top trace is instantaneous firing rate and bottom trace is vertical eye position. (B) Mean firing rate during fixation period between saccades (tonic eye position component) as a function of eye position for all four conditions in (A). The slope of each line gives the eye position sensitivity of the neuron for each condition. Colors of the points and lines correspond to the headings above each panel in (A). Green, control (no injection); Purple, bicuculline; Red, GABA; black, bicuculline and GABA.

and another containing the GABA-A antagonist bicuculline. This neuron showed a typical burst-tonic response during spontaneous saccadic eye movements while the drugs were retained during the control period (Fig. 6A), consistent with the INC's role as part of the velocity to position neural integrator. When bicuculline was injected in isolation (Fig. 6B), the neuron responded by substantially increasing the tonic eye position component of its firing rate, resulting in a higher baseline firing rate. This increase resulted in a shifting of the firing rate outside the normal range of the neuron. Injection of GABA had the opposite effect, reducing the overall firing rate range of the neuron (Fig. 6C). Paired injection of bicuculline and GABA brought the neuron firing rate back within its normal range (Fig. 6D), but the tonic eye position component of the firing rate, represented as the slope of the lines in Fig. 6E, was higher than during the control period (compare black line in Fig. 6E with green line). This indicates a specific effect of bicuculline on the neuronal eye position sensitivity that is not due to an increased overall responsiveness.

These example recordings indicate that our multibarrel electrodes are capable of recording from diverse areas deep within the primate brain. More complete characterizations of the neuronal responses to pharmacological manipulation will be presented in subsequent papers.

4. Discussion

We have presented a detailed description of a fast and effective procedure for manufacturing multibarrel electrodes that use a carbon fiber for single unit recording. These electrodes can be used for experiments conducted in a variety of deep structures in the alert behaving primate. We have employed this technique with great success for the past 3 years and have not seen any increase in the number or degree of encephalic infections in our monkeys, indicating that the technique is suitable for recording in chronically implanted animals. The long, thin tapers of these electrodes easily fit inside a 25 gauge guide tube, which ensures limited tissue damage during repeated penetrations. In addition, we performed a parametric study of a variety of multibarrel configurations, including three, four, and seven barrels of different lengths and salt concentrations. This should aid investigators wishing to apply the technique to their own unique experimental needs.

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