A Multi-Channel, Implantable Microdrive System for Use With Sharp, Ultra-Fine “Reitboeck” Microelectrodes

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INTRODUCTION

It is now commonplace for acute electrophysiological experiments to be conducted on chronically prepared subjects for periods of many months. In some such experiments, the aim is to record simultaneously from groups of well-isolated single neurons, and closely spaced arrays of independently controlled “Reitboeck” electrodes (Reitboeck 1983a) have proved to be especially well suited for such aims (e.g., Alonso et al. 2001; Baker et al. 1999; DiCarlo et al. 1998; Eckhorn et al. 1988; Gail et al. 2004; Lee et al. 1998; Mountcastle et al. 1991; Steinmetz et al. 2000; Swadlow and Gusev 2001, 2002; Usrey et al. 2000). These electrodes are fabricated from filaments consisting of a platinum-alloy core (platinum: 90%, tungsten: 10%) and an insulating shell made of quartz. Because the metal alloy core and the quartz insulation have similar melting points and expansion coefficients, these filaments can be pulled under high temperature to a fine taper. The tips are then ground to the desired characteristics. The fine and consistent outer diameters of these filaments (commercially available at 80, 60, and 40 μm, Thomas Recording, Giessen, Germany) allows them to be fit into guide tubes that direct them to a very small area of brain tissue, and the stiffness of these filaments generates a predictable electrode trajectory. To manipulate these fine-diameter electrodes, microdrive systems have been developed that allow independent control of each electrode while preventing buckling of the portion of the narrow shafts that lie above the guide tubes (Reitboeck 1983a,b; Eckhorn and Thomas 1993; see reviews by Baker et al. 1999; Mountcastle et al. 1991). These methods require the electrodes to be inserted into the brain (usually through the dura) at the beginning of each recording session, manipulated by the external microdrives, and removed at the end of a recording session.

For some experimental aims, however, it would be desirable to leave electrodes within the brain for longer periods and to slowly explore each of the single neurons that lie along several closely spaced microelectrode penetrations. In principal, Reitboeck electrodes are well suited for chronic indwelling use because both the platinum alloy core and the quartz shell are very durable and stable materials. Here, we describe a system for using densely packed, indwelling arrays of independently controlled Reitboeck electrodes for long-term, chronic recording. We describe a compact and simple method for preventing the buckling of these electrodes that is suitable for use with electrode shaft diameters as small as 40 μm, an ultra-miniature, multiple microdrive system that allows the independent control of these electrodes, and procedures for keeping electrodes mobile and under microdrive control for many months by minimizing the entrance and subsequent coagulation of brain fluids within the guide tubes. We have used variants of this system in rabbits for >5 yr. Electrodes typically remain mobile, under microdrive control, and able to record from well-isolated single neurons for periods of many months and, in once case, for >4 yr.

METHODS

The methods in the following text were developed to accommodate Reitboeck electrodes constructed of filaments of 40 μm OD (metallic core of 14 μm, Thomas Recording) but can be scaled up to accommodate the larger filaments (80 μm OD) as well. These electrodes are pulled to a fine taper in a high-temperature (~2100°C) vertical puller (Thomas Recording). This is done within a chamber that is filled with inert gas to prevent oxidation of the tungsten filament. The tips are then sharpened to the desired characteristics using a fine diamond
Center, OH). The upper portion of the guide tubes are separated and diamond grinding wheel (0.1 mm thick, Abrasive Technology, Lewis and beveling of stainless steel tubing is accomplished using a small slightly beveled and cleaned after construction is complete. Cutting ends are bound together in a concentric array by tight-fitting polyimide thinned to 0.3 revolutions/s) using a toy motor that drives the microdrive near the nut.3 The microdrive is light (made from 22-gauge, thin-wall stainless steel tubing, Small Parts) and shows its basic design principle. A fine stainless steel screw (0000–160, J.I. Morris, Southbridge, MA) is housed within a cylinder (made from 22-gauge, thin-wall stainless steel tubing. Small Parts) that has a nut (matching the 0000–160 screw) soldered to the top. A cross-bar is soldered to the top of the screw so that it can easily be turned. The screw is threaded through the nut, and its distal, flattened tip pushes down on a piston, which has an armature that exits the cylinder at approximately a 90° angle via a groove along the length of the cylinder. This groove serves to constrain the motion of the armature to a single (z) axis. The armature will later be secured to the microelectrode and will control its motion. The piston is held tightly against the base of the screw by a small compression spring that puts an upward pressure on the armature. The microdrive is light (~60 mg) and is narrow enough (~1 mm diameter) so that microdrives can be spaced at distances of ~1.3 mm.

Preventing blockage of the guide tubes

It is crucial that fluids from the brain be prevented from entering the guide tubes. Otherwise, solidification of the fluids would soon prevent the movement of the electrodes. To deal with this problem, we first fill each guide tube with a single application of a sterile antibiotic ointment (Vetropolyvin, a bacitracin-neomycin-polymyxin opthalmic ointment). This is accomplished by pressure injection, until the ointment can be seen emerging from the distal ends of the tubes. Next, we create a hydrophobic seal at the tips with melted bone wax. The microelectrodes readily penetrate a few hundred micrometers of the bone wax, but the wax must be securely positioned and held within/over the tips of the guide tubes. Otherwise, the electrodes could displace rather than penetrate the wax. We have used several means to achieve this. Most recently, for studies of cortex, we have secured a length of polyimide tubing over the guide tube array (using Vetbond, a biocompatible cyanoacralate glue) to create a space at the tips of ~0.25 mm. After filling the guide tubes with antibiotic ointment, with the electrodes retracted within the guide tubes, the space at the tip of the guide tube is filled with melted bone wax (~40 nl of bone wax is required to fill this space). As shown in Fig. 1B, the microelectrodes readily penetrate this amount of bone wax.

Microdrives

Many systems of simple, screw-driven microdrives have been developed for chronic recording purposes. To be suitable for the preceding electrode-guide tube arrays, the microdrive must simply provide the appropriate linear motion along the z axis (3–6 mm for our purposes) and be narrow enough to allow one microdrive to be aligned with each guide tube without overcrowding. The requirement for such close spacing of microdrives presents a challenge. Two components are generally found in screw-driven microdrives: a screw and a linked shaft assembly, located parallel to the screw, that constrains motion to a single (z) axis. To reduce the x-y dimensions of the microdrive so that drives could be more densely packed, this general design principle was modified and the parallel shaft assembly was eliminated. Figure 3 is a schematic illustration of this microdrive and shows its basic design principle. A fine stainless steel screw (0000–160, J.I. Morris, Southbridge, MA) is housed within a cylinder (made from 22-gauge, thin-wall stainless steel tubing. Small Parts) that has a nut (matching the 0000–160 screw) soldered to the top. A cross-bar is soldered to the top of the screw so that it can easily be turned. The screw is threaded through the nut, and its distal, flattened tip pushes down on a piston, which has an armature that exits the cylinder at approximately a 90° angle via a groove along the length of the cylinder. This groove serves to constrain the motion of the armature to a single (z) axis. The armature will later be secured to the microelectrode and will control its motion. The piston is held tightly against the base of the screw by a small compression spring that puts an upward pressure on the armature. The microdrive is light (~60 mg) and is narrow enough (~1 mm diameter) so that microdrives can be spaced at distances of ~1.3 mm.

Preparing the microdrive system for use

Prior to use, the guide tube array is disinfected in 70% alcohol. Because liquids do not readily enter such fine tubing, the alcohol is

1 We grind electrode tips using a continuously wetted, “extra fine” diamond abrasive plate (Sutter Instrument, Novato, CA, plate model 104 F), that is rotating toward the electrode tip (at 1–3 revolutions/s) on a homemade horizontal turntable. The electrode is held within a length of stainless steel tubing at an angle (to the grinding wheel) of ~12°. During grinding, the electrode is rotated about its axis (at ~0.3 revolutions/s) using a toy motor that is linked to the electrode by a rubber band. A complete grinding system is commercially available from Thomas Recording.

2 Another means to control the position of the guide tubes is to fit them into slots that are ground into the side of the template (rather than into drilled holes). It is easier to fit the guide tubes into these slots, but the upper ends of the guide tubes are generally more divergent when using this method.

3 An alternative means of maintaining upward tension on the piston is through the use of a very fine band of elastic material that pulls upward on the armature and is attached to the microdrive near the nut.
pressure injected into each tube and then placed within the bath. After disinfection, the guide tubes are filled, by pressure injection, with the antibiotic ointment, and melted bone wax is applied to the tips of the guide tubes to create the hydrophobic seal. The electrodes are usually inserted and attached to the microdrives prior to the day of surgical implantation. Electrodes are inserted into the guide tubes under microscopic control and manually advanced until the polyimide tubing over the upper portion of the electrode passes over the stainless steel guide tube. The upper portion of the electrode (the polyimide tubing) is then secured to the microdrive armature using a small amount of melted dental impression compound (illustrated in Fig. 3J, right). This juncture is rigid but can be readily broken to re-position or exchange an electrode (electrodes can be exchanged either before or after the array has been implanted). The electrode is then advanced using the microdrive until the electrode tip is observed to emerge from the bone wax at the tip of the guide tubes. The electrode is then retracted a known distance (100 or 200 \( \mu \)m) into the bone wax. This procedure is repeated for each of the seven electrodes. The electrodes can also be placed into the guide tubes after the array has been surgically fixed into position, but the position of the electrode tips are less accurately known when using this procedure.

**Implanting the array and fixing it to the skull**

The array is mounted on a stainless steel rod that can be held by a stereotaxic carrier. The rod has a break-point near the junction with the array so that it can be released after being cemented to the skull. For cortical recordings, the bone wax at the tips of the guide tubes is placed in contact with the dura. For thalamic recordings, we usually insert the guide tubes through overlying (nonrelated) cortex so that the tips lie 3–4 mm above the region under investigation. Antibiotic ointment is applied to the surface of the dura, and the array is cemented into place using acrylic cement. This cement is applied liberally over the skull and around the guide tubes and is joined to the cement around the head-bolt assembly to ensure stability. Further stability is achieved by creating a cement bridge that joins the acrylic mass on the skull to the position on the array where the guide tubes are beginning to separate (10 mm above the skull). Electrical contact between the electrodes and a miniature plug is made by soldering a length of Teflon coated Platinum-iridium wire (25 or 50 \( \mu \)m core diameter) to the platinum rod at the top end of each electrode. Our seven-channel microdrive system (as shown in Fig. 1) generally extends 30–35 mm above the level of the skull. A molded plastic cap that attaches to the head bolt protects the array between recording sessions.

**RESULTS**

This system, or similar prototypes, has been in use for >5 yr in studies of the somatosensory and visual systems of the awake rabbit. Recordings were obtained from neurons of ventrobasal thalamus (VB thalamus) (Swadlow and Gusev 2000–2002; Swadlow et al. 2002), dorsal lateral geniculate nucleus (LGNd) (Bezdudnaya et al. 2003; Cano et al. 2003), visual and somatosensory cortices, and the superior colliculus. Figure 4, A–C, shows light and electron micrographs of three of seven electrodes that had been used to record from the LGNd of one rabbit for a little more than 6 mo. Importantly, six of the seven electrodes in this array remained mobile for this entire period. No deterioration of the tips is visible at this degree of magnification. A final exploratory penetration within the LGNd was made 1 day prior to removing these electrodes from the brain using the electrode shown in Fig. 4C. Figure 4D, 1–4, shows spikes, associated clusters, and visual

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4 One of the seven microdrives ceased to function due to an inadequate solder joint between the nut and the stainless steel tube (Fig. 3).  
5 The bent tip in electrode “C” is clearly the result of a mechanical accident. We do not know when this occurred but, based on the fresh appearance of the fractured quartz in the electron micrograph (Fig. 4C2), we guess is that it probably happened after these recordings were obtained as the electrode was removed from the guide tube.
recordings were periodically obtained with electrodes within and above VB thalamus, for a period of 4 yr and 5 mo. This subject was studied extensively during the initial 1-yr period, and recordings were periodically obtained by the electrode tip, often by "buckling. Dimensions given are for use with guide tubes 22 mm in length. A: pulled and sharpened electrodes are cut to a length of 31 mm, and the quartz at the rear end of the electrode is broken such that a few hundred micrometers of metal core extends from the upper end (→). B: electrical contact with the fine platinum-tungsten core is achieved by soldering a 3-mm length of platinum rod (yellow, 200–250 μm diam) to the core so that ~1 mm of platinum rod overlaps with the quartz insulation. The mechanical junction between the quartz and the platinum rod is reinforced with cyanoacrilate cement. C: the electrode is threaded through a 7-mm length of polyimide tubing (red, 250 μm ID, 300 μm OD, A-M Systems). The inner diameter of this tubing must be somewhat greater than the outer diameter of the stainless-steel guide tubes (gray) that are used. This tubing is gently fitted around the platinum rod to reach a few hundred micrometers beyond the solder juncture of the platinum rod and the electrode core. The polyimide tubing serves 2 functions: it further strengthens the mechanical junction between the electrode and the platinum rod and it prevents buckling of the electrode as it is lowered (following text). D: the electrode can now be threaded into 1 of the guide tubes. Once the polyimide tubing passes over the top of the guide tube, the upper portion of the electrode shaft is no longer subject to buckling as it is lowered (E).

During the subsequent years. At the end of this period, three of the seven microelectrodes were still under microdrive control and were recording well. Moreover, neurons with apparently normal receptive fields were still encountered by these electrodes (i.e., most of the neurons yielded robust responses that were dominated by a single vibrissa and showed a clear preference for the direction of vibrissa displacement) (e.g., Swadlow and Gusev 2002). Figure 5, A and B, shows micrographs of two of these electrodes, and C shows action potentials (and associated cluster analysis) that were recorded from the electrode shown in A. These recordings were obtained at several depths within VB thalamus, just before these electrodes were removed from the brain. Clearly, there is some deterioration of the quartz insulation that is visible near the tip. Figure 5, A2 and B2, shows electron micrographs of these electrode tips that show, more clearly, the deleterious effects of >4 yr within the brain.

To quantify this deterioration, we measured the diameter of these electrode shafts at a distance of 2 mm from the tip (where the shaft had been in contact with the brain) and at a distance of

7 By the end of the initial year after implantation, one of the seven microdrives was broken and another of the electrodes was frozen within its guide tube. By the end of the fourth year, two additional electrodes were immobile, but three of the seven electrodes could still be moved and were fully functional.

These receptive fields were concentrically organized and only the on-center responses are shown. The fields (and those in Fig. 7) were generated using methods of reverse correlation. Stimuli consisted of 1 or 2° flashing light spots, presented pseudo randomly in a spatial grid of ≥16 × 16.
15 mm from the tip (where the shaft had been protected within the guide tube). For both electrodes, the shaft was reduced in diameter by 4–5 μm at the distal site. This value, and the visible characteristics of the tips, are consistent with a dissolution of the quartz insulation from the surface of the shaft at a rate of ~0.5 μm/yr.

The rabbit described above was killed at the age of 5.75 years, after the guide tube array had been in place for 4 yr and 5 mo. The rabbit was perfused with saline followed by formalin, and the tissue was sectioned and stained for Nissl substance. This array was implanted a little deeper than we had...
planned, and the tips of the guide tubes inadvertently penetrated the dorsal thalamus (Fig. 6A). Unfortunately, the preparation of this tissue was less than ideal, the plane of our tissue sections were not well-aligned with the angle of the guide tubes, and many freezing (and other) artifacts are present. Nevertheless, aside from the mechanical damage done by the guide tubes (total outer diameter of the array was \( \sim 0.5 \) mm, as in Fig. 1B), damage to tissue around the tips of the guide tubes (which had been filled with bone wax) seemed minimal. No unambiguous signs of the electrode tracks could be followed to VB thalamus (Fig. 6B), where recordings were obtained.

For cortical recordings, and for recordings from the superior colliculus (which lies \( \sim 5 \) mm beneath the dural surface of the rabbit), the tip of the guide tube array rested on the dural surface. Because extensive tissue reaction and growth often occurs on the dura, we were concerned that the guide tubes might be quickly blocked, with a consequent loss of electrode mobility. This occurred only very rarely. Figure 7 (top) shows receptive fields generated in superficial superior colliculus by five microelectrodes within 1 wk after this electrode array was implanted. These electrodes were of low impedance (<1 M\( \Omega \)), designed to be optimal for multunit and field potential recordings, as well as for microstimulation. Figure 7, bottom, shows the receptive fields recorded from these same electrodes just >1 yr later. Receptive fields were periodically monitored during the intervening months, and between these measures, the electrode tips were usually moved to a position \( \sim 1 \) mm above the collicular surface.

**Discussion**

Reitboeck electrodes have a number of characteristics that make them very well suited for long-term use within the brain: 1) the platinum-tungsten alloy core has excellent recording characteristics and is virtually inert. In contrast, electrode tips made of pure tungsten (which is not inert) can degrade considerably after characteristics and is virtually inert. In contrast, electrode tips made of pure tungsten (which is not inert) can degrade considerably after 4 yr in the brain (Malpeli et al. 1992). 2) The quartz insulation is very tough and, relative to other glasses, is very stable. The degradation that we noted after >4 yr in the brain is consistent with a dissolution of the surface of the quartz insulation at a rate of \( \sim 0.5 \) \( \mu \)m yr\(^{-1} \). And 3) the very fine maximal outer diameter of the Reitboeck electrodes allow them to be funneled down very fine-diameter guide tubes for very close electrode spacing, yet their stiffness generates a straight and predictable electrode trajectory.

In all of these experiments, the rabbits were awake, but the head was fixed during recordings. Thus these were not freely behaving subjects, and our experimental aims have generally been to record from well-isolated single neurons for several hours and then to move on to record from other such neurons. Because these electrodes can be fabricated with very fine tips, they are well suited to record from neurons of all sizes. Although long-term recordings from the same neuron was not the goal of these experiments, we sometimes did record from a neuron for several days (e.g., see neuron “Hercules” in Swadlow and Gusev 2002). Low-impedance, fixed microwires are undoubtedly better suited when it is desirable to study the same neuron for very long periods of time (e.g., Porada et al. 2000; Swadlow 1982, 1985).

We have used different variations of this system in rabbits for >5 yr and have varied the seven-channel concentric design described here in several ways. For cortical recordings, we have employed three-channel triangular arrays, seven-channel concentric arrays, or five to seven channel linear arrays of guide tubes and electrodes (most with 150-\( \mu \)m spacing as described in the preceding text). We have also employed 19-channel\(^8\) concentric arrays of guide tubes and electrodes for

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**Fig. 7.** Top: multi-unit receptive fields generated in superficial superior colliculus by 5 microelectrodes 1 wk after this electrode array was implanted. These electrodes were of low impedance (<1 M\( \Omega \)) and designed to be optimal for multunit and field potential recordings, as well as for microstimulation. Bottom: receptive fields recorded from these same electrodes 12 mo later. Recordings were obtained several times during the intervening months, and electrodes were periodically moved to various positions within and above the colliculus. At the end of the 1-yr period, all of the electrodes were still mobile and, when re-positioned in superficial collicular layers, yielded receptive fields with relative spatial positions that were very similar to those initially recorded.

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\(^8\)Due to geometric constraints, concentric arrays of 7 or 19 channels (with 1 ring or 2 rings of guide tubes, respectively, around the center tube) are most easily fabricated when all guide tubes are of the same outer diameter.

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**Fig. 6.** Nissl sections from 1 rabbit in which the guide tube array had been in place for 4 yr and 5 mo. This array was implanted a little deeper than we had planned, and the tips of the guide tubes inadvertently penetrated the dorsal thalamus (A). Many freezing (and other) artifacts are present but, aside from the mechanical damage done by the guide tubes, tissue damage around the tips of the guide tubes (which had been filled with bone wax) seems minimal. B: a section taken through ventrobasal thalamus (VB thalamus, dashed outline), where recordings were obtained. This section is \( \sim 1.8 \) mm posterior of that shown in A (because the plane of section is misaligned with that of the guide tubes). No unambiguous signs of the electrode tracks or tissue damage could be followed to this nucleus. However, it is possible that some damage above VB thalamus may be related to the microelectrode penetrations (arrow). The calibration bars in A and B = 1 mm.
recording both from the thalamus and from the superior colliculus. Somewhat longer guide tubes (27–30 mm) are required for such large arrays to allow adequate spacing for the microdrives.

To prevent fluids from entering and solidifying within the guide tubes, we used small quantity of bone wax to create a hydrophobic seal at the tip of the guide tube. In our initial studies, we sealed the tips of the guide tubes so that a small amount of hot bone wax entered the tubes. In later studies, we expanded this hydrophobic seal by creating a wax-filled chamber, containing ~40 nl of bone wax, at the tips of the guide tubes (as in Fig. 1B). Whereas bone wax is relatively biocompatible, there are some reports of long-term effects of this substance (Aksu et al. 2001; Alberius et al. 1987; Allison 1994). Moreover, bone wax is resorbed, albeit very slowly. Undoubtedly, there are better ways of creating a hydrophobic seal at the guide tube-brain interface that is penetrable by fine microelectrodes. Of course, it is generally prudent to position the tips of the guide tubes as far as possible from the tissue under investigation (e.g., on the surface of the dura when this is possible).

Chronically implanted fixed microwires can remain within the cortex and, in some cases, record from the same neurons for periods in excess of 1 yr (e.g., Porada et al. 2000; Swadlow 1982, 1985). For many experimental aims, however, microwires do not yield optimal recordings. The present work presents a system that enables the use and control of fine-wires without optimal recordings. The present work presents a system that enables the use and control of fine-wires without optimal recordings.

G R A N T S

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R E F E R E N C E S


