Multichannel Micromanipulator and Chamber System for Recording Multineuronal Activity in Alert, Non-Human Primates

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This problem, the dura must be repeatedly “thinned” to avoid electrode damage (Spinks et al. 2003) and excessive dimpling. This procedure risks further infection, is time consuming, and is stressful to the animal. Finally, daily implantation of one or more guide tubes through the dura can result in significant damage, potentially cause the animal pain, or further increase the possibility of infection.

The principal problems with currently available micromanipulators are that they are usually limited to one or a few electrodes, and they often employ bulky and/or manual mechanisms for controlling electrode movement, can be difficult to electrically shield, or are quite expensive, putting them beyond the resources of many laboratories (Mountcastle et al. 1991; Reitboeck and Werner 1983). These problems are exacerbated if the experimental questions require monitoring of neuronal activity from more than one region of the brain.

Here we describe the design and performance of a new type of recording chamber and micromanipulator system that can solve or reduce these problems and increase the feasibility for multineuron recording from one or more regions of the brain simultaneously. The chamber system is small, is low in profile, and provides a semi-chronic window onto the brain through the use of a watertight, replaceable Sylastic membrane (Arieli et al. 2002). The membrane is self-sealing, prevents infection and allows multiple electrodes to be easily and repeatedly introduced. The micromanipulator allows the remote control of eight independently moveable microelectrodes, is well shielded and easy to setup and use, and is comparatively inexpensive. The control electronics can operate up to eight micromanipulators at a time, making it feasible to monitor neuronal activity from multiple regions of the brain simultaneously.

METHODS

All mechanical design work was performed using SolidWorks, a computer aided design package (SolidWorks). Electrical circuit design and the layout of printed circuit boards were performed using the OrCAD Capture and OrCAD Layout software packages, respectively (Cadence).

Chamber system

The chamber system, shown in Fig. 1. was designed with two objectives in mind. First and foremost, we wanted the intracranial space to be hermetically sealed off from the external environment. This would serve to reduce the chances of infection and help reestablish the normal balance of intracranial pressure. The barrier for maintaining the seal had to permit the passage of microelectrodes without disrupting the seal or damaging the electrodes and ideally

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Innovative Methodology

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would lie in direct contact with the surface of the dura. To satisfy both constraints, we chose to use a thin membrane of Sylastic (Dow-Corning, No. 7–4107) that had been successfully employed in optical imaging experiments in non-human primates (Arieli et al. 2002). While allowing easy passage of the electrodes, the membrane’s ability to reseal is limited to a relatively small number of electrode penetrations (5–10 at a single site). It therefore became necessary to make the membrane replaceable after its seal becomes compromised. Our second objective was to keep the diameter and height of the chamber small, so that multiple chambers could be implanted in a single animal and so that the dural or cortical surface of the brain could be easily accessed during simple surgical procedures.

To accomplish these objectives, we designed the system to consist of five components. Design drawings of the system are shown in Fig. 1A and a completed system is shown in Fig. 1C. The components include the chamber, a removable sleeve and retaining ring, a plug, a cap, and an O-ring. The chamber, sleeve, and retaining ring are constructed from Titanium (6AL/4V ELI), and the plug and cap are constructed from Ultem plastic. The O-ring is made of silicone 70 durometer red and is manufactured by Sterling Seals.

To bring the Sylastic membrane in contact with the dural surface, we first designed the chamber to have a lower extension of its wall that would fit snugly within a circular craniotomy (Fig. 1B). The depth of the lower wall was set to 2 mm so that when implanted its bottom surface would rest gently on the surface of the dura. Depending on the location of the implant, this often required some thinning of the cranial bone to ensure even thickness around the chamber. The sleeve was designed to thread into the chamber, and its vertical dimension adjusted so that its bottom surface would lie flush with the bottom of the chamber wall. To ensure a tight seal between the chamber and the sleeve, we introduced a groove on the chamber’s upper surface for placement of an O-ring, and a flange on the upper surface of the sleeve that would overlap the upper wall of the chamber. When the sleeve is fully threaded into the chamber, its flange exerts downward pressure on the O-ring generating a water-tight seal. To attach the membrane to the bottom surface of the sleeve and thus bring it into contact with the dural surface, the outer diameter of the sleeve was stepped down so that a small retaining ring could be easily placed over the end (Fig. 1A). This allows the membrane to be mounted by laying it across the bottom surface of the sleeve and then press fitting the retaining ring in place (Fig. 2). When done in this way, the membrane is stretched across the bottom of the sleeve like a drum head. The residual Sylastic can then be removed with a scalpel. Finally, when not in use, the lumen of the chamber is filled with the plug and covered with the protective cap. This provides an abutting surface, similar to the cranium, so that changes in intracranial pressure don’t lead to protrusion of the brain through the craniotomy. In early versions of the design, we noticed that the insertion of the sleeve often trapped a bubble underneath the membrane, and in one case, this was sufficient to compress the cortical circulation and produce a permanent lesion. To avoid this problem, we introduced a set of four vertical grooves on the inside wall of the chamber, and a central hole in the plug, to allow air to escape when either the sleeve or the plug are mounted within the chamber.

To provide consistent positioning of the manipulator within the chamber and make the sleeve easy to remove, the upper surface of the sleeve was designed with three small, closed-end holes (Fig. 1C). The smaller of the three holes provides for placement of a centering pin on the manipulator, and the remaining two symmetrically placed holes allow for the insertion of a tightening tool. The tool proved to be necessary to obtain a tight seal with the O-ring and to enable the removal of a tightly placed sleeve. Finally, the chamber was designed with an external groove on its outer wall so that acrylic cement could be used to fix the chamber to the skull in combination with Titanium orthopedic bone screws (Synthes). In future versions of the chamber, we plan to incorporate a perforated flange on the bottom of the outer wall that will enable the chamber to be mounted directly with bone screws and eliminate the need for acrylic cement.

**Mechanical components of the manipulator system**

**ASSEMBLED SYSTEM.** The micromanipulator was designed to couple to the recording chamber, and provide remote control of eight independently movable microelectrodes. The mechanical components consist of a set of eight linear actuators mounted in a machined aluminum block that is fixed to a frame assembly. Design drawings of the system are shown in Fig. 3, and a completed system is shown in Fig. 4. When assembled, the manipulator consists of seven mechanical and structural components and two printed circuit boards (PCB, see following text). The actuator block houses a set of eight linear
actuators, the base cone holds the guide cylinder (described in the following text), and the block and base are held together by four support arms. A pair of standoffs allow the motor control PCB to be positioned above the actuator motors, a plastic cap covers the top of the assembly, and two side covers enclose the interior. All of the metal components are made of Aluminum, were machined on a CNC milling machine, and anodized to provide a protective surface.

When fully assembled (Fig. 4A), the system is 5.63-in (143 mm) in height and weighs 129.5 g. The outer diameter of the chamber and the protective cap are 0.775 in (19.7 mm) and 1.425 in (36.2 mm), respectively. The inner diameter of the chamber sleeve is 0.5 in (12.25 mm).

LINEAR ACTUATOR. A photograph of a completed linear actuator is shown in Fig. 5. The device consists of a miniature stepping motor (5 mm OD) fitted with a four-conductor flex-print cable, a 1:125 gearbox, a lead screw (100 threads/in), a plunger, and an electrode holder. The motor, gearbox, cable, and lead screw were purchased from MicroMo (No. BL.2S5.125.L.0). The plunger is constructed from Ultem-1000 plastic and has two grooves machined along opposite sides of its long axis (Fig. 5, inset), a set of internal threads matching those on the lead screw, and a circular hole on the opposite end for mounting the electrode holder. One of the grooves is flat and provides for the placement of a thin Beryllium-Copper (BeCu) strip (0.772-in L, 0.06-in W, 0.002-in thick; Italix) using cyanoacrylate cement. The strip extends over the full length of the plunger and provides for electrical contact between the electrode holder and the signal PCB when the system is fully assembled. The other groove in the plunger is semi-circular and meshes with a cylindrical, stainless steel key pin that fits into the actuator block. When assembled, this mechanism ensures that the rotation of the lead screw is converted to a linear translation of the plunger during activation of the stepping motor. The electrode holder is made of Brass and is mounted on the end of the plunger using a press fit. When mounted, the upper surface of the electrode holder comes into firm contact with the bent end of the BeCu strip. The electrode holder has a beveled hole in its bottom surface for back loading the electrode, and a pair of threaded holes to

FIG. 3. Exploded view of the design drawings of the microdrive system revealing all component parts and their relative positions within the device.

FIG. 4. Photographs of the fully assembled microdrive (A) and a second system with the covers and chamber system removed (B). Note the intermediate guide mounted onto the interior of the support arms in B.

FIG. 5. Photograph of a fully assembled linear actuator. The colored pictures to the right show design drawings of the plunger, illustrating the 2 types of slots machined into the body of the plunger.
mount opposing set screws (1/16 in, 0–80) that enable the electrode to be held firmly in place. The bottom surface of both set screws are ground flat to minimize shearing forces to the end of the electrode.

**ACTUATOR BLOCK.** A completed actuator block is shown in Fig. 6. The device is machined from a block of aluminum and anodized to protect its surface from scratching and provide electrical insulation. It contains a set of eight holes, drilled at an inward angle of 7°, arranged in a circle for mounting each of the linear actuators. Each hole has two inner diameters, one for mounting of the actuator gearbox (Fig. 6A) and a second that allows for smooth passage of the plunger (Fig. 6B). An additional slot is milled into the inner edge of the bottom portion of each mounting hole to allow for placement of the key pins. Two threaded holes on the top of the block allow for mounting of standoffs to hold the motor control PCB, and three threaded holes on the bottom of the block allow for mounting of the signal PCB. Four slots, with threaded holes, allow for mounting of the support arms to the bottom portion of the block, and a central threaded hole on the bottom surface allows a circular disc to be mounted for retaining the key pins within the block.

**BASECONE, GUIDE CYLINDER AND RETAINING CAP.** The lower portion of the assembly consists of two components, the basecone and the guide cylinder (Figs. 3 and 7). The basecone provides the structural framework for linking the actuator block to the guide cylinder. Its upper portion contains slots for mounting the support arms, and its lower portion contains a threaded receptacle for mounting the guide cylinder (Fig. 7A). The guide cylinder has several key features that facilitate the alignment of electrodes and the mounting of the microdrive to the recording chamber (Fig. 7B). First, it is designed so that a guide array can be press fit into its bottom opening. The tight fit ensures that it will not move or rotate within the cylinder. The guide array is a cylindrical block of Ultem-1000 plastic (0.5-in height) containing an 8 × 8 array of parallel guide holes [0.015 in (0.37 mm) ID, 0.035 in (0.86 mm) spacing] that have been drilled through its long axis. These holes serve as guide tubes for positioning electrodes within the chamber. The bottom end of the guide array is flanged so that it always aligns to the same vertical position when mounted in the cylinder. Second, the guide cylinder is constructed with a flange located approximately one-third of the distance from its bottom surface, and a guide pin is press fit into the lower surface of the flange. When the system is mounted onto a recording chamber, the flange ensures that the bottom surface of the guide array will come to rest at a position slightly beyond the plane of the Sylastic membrane. This ensures a tight apposition between the guide array and the membrane. The guide pin ensures that the guide array will always be mounted at the same position within the recording chamber. Finally, the flange on the guide cylinder also enables the incorporation of a retaining cap, which attaches the microdrive to the recording chamber (Figs. 3 and 4). This cap is mounted onto the guide cylinder before it is threaded into the basecone, and its inner surface contains threads that match those on the outer surface of the recording chamber. When the cap is threaded onto the chamber, it exerts downward pressure on the guide cylinder flange and ensures a tight, vibration free fit between the microdrive and the chamber.

**PRINTED CIRCUIT BOARDS.** The manipulator is designed to house two PCBs. One board provides connections to the motor control circuitry (see following text) and another to the amplifier headstage. Both boards were manufactured by Trilogy-Net, and are shown in Fig. 8. The motor control PCB (A and B) is a simple two-layered board that provides connections between each of eight four-pin connectors (Molex, No. 52808×0490) on the bottom surface (B) to a single 32-pin, zero-insertion-force, connector (Molex, No. 52559×3292) on the top surface (A). Connections are made to the latter connector from the motor control system via a 32-conductor flex-print ribbon cable (Parlex, No. 050×32×1524B).

The second board, shown in Fig. 8, C and D, is designed to make contact to each of eight electrodes via a spring-loaded sliding contact mechanism. To accomplish this, the board was built in a circular configuration with solder pads arranged in register with the plungers.

**FIG. 6.** Photographs of a completed actuator block as viewed from the top (A) and bottom (B).

**FIG. 7.** Photographs of a completed basecone (A) and guide cylinder (B). The guide cylinder is shown with (right) and without (middle) a mounted guide array (left).
of each actuator. Electrical contact is established by soldering a short beryllium-copper (BeCu) tab (0.120 × 0.040 × 0.002 in) to each pad so that it extends 2 mm toward the center of the circuit board (Fig. 8, C and D). This ensures that when the PCB is mounted onto the actuator block, each BeCu tab is gently pressing against the corresponding BeCu strip of the plunger thereby making a spring-loaded contact.

The BeCu tabs and strips were made by Italix (Santa Clara, CA). They were cut, bent, heat treated, and cleaned. The tabs were soldered onto the PCB during the final step in manufacture of the boards (Trilogy-Net). The connector making contact with the amplifier headstage consisted of nine pins having a pitch of 0.05 in. This connector was compatible with MultiChannel Systems headstage (MPA81); however, the connector can be easily replaced to accommodate other types of headstage amplifiers.

**Assembly**

For assembly, the lead screw on each actuator is fitted with a plunger and electrode holder (Fig. 5) and then mounted into one of the holes in the actuator block (Fig. 6A). The flexprint cable attached to each motor is folded upward and inserted into the corresponding connector on the underside of the motor control PCB (Fig. 8B). The PCB is then fixed to the standoff that are mounted on the top surface of the actuator block. The key pins are then placed into each hemispherical slot on the underside of the actuator block, and the retaining plate is screwed in place. Once this step is completed, the signal PCB is gently placed over the ends of the plungers and fixed in place with cap screws. During this step, it is important to insure that the BeCu tabs do not catch on the ends of the plungers or electrode holders, otherwise they will be bent backwards and will have to be repaired or the PCB replaced. Figure 9 shows a bottom-up view of an assembled microdrive before the support arms and basecone are attached. B shows a magnified view of the sliding contact between the BeCu tabs on the signal PCB and the BeCu strips mounted on each plunger. C shows the relationship between the key pins mounted within the actuator block and the semicircular groove in each plunger. In the final steps of the assembly, the support arms are mounted onto the basecone and the actuator block. The retaining cap is placed over the guide cylinder, which is then threaded into the basecone (see Figs. 3 and 4 for details).

**Motor control system**

The control system that interfaces to the micromanipulator consists of two main parts, a rack-mounted electronic motor controller (EMC), and a remote motor interface unit (RMIU). A schematic diagram of the control system architecture is shown in Fig. 10, and photographs of the printed circuit boards are shown in Fig. 1 of the supplementary materials. The main component in the EMC is the Atmel ATmega128 8-bit RISC microcontroller operating at 14.7456 MHz. This device communicates with a monitoring and control computer via a serial connection, stores the positions of all the motors, and provides the control signals for the timing of the stepping motors. Software for the microcontroller was written in C, converted to assembly code and downloaded using the software package AVR Studio 4 (Atmel). The serial communication protocol utilizes ASCII command strings, which are newline character terminated. Error detection is accomplished using an odd-parity bit. The high level serial (RS-232) signals are level shifted to the I/O voltages required by the microcontroller using a MAX232 interface chip.

The RMIU controls motor selection and delivers current to the selected motor. Each unit houses eight removable motor control cards, each capable of driving eight stepping motors. The cards are mounted in a backplane card, capable of mounting eight control cards to provide control of ≤64 motors (Fig. 1, B and D, supplementary material). Each control card has its own shift register (SN74HC595D, Texas Instruments), to enable motor selection, and a single N-channel (IRF7456) and three P-channel MOSFETs (IRF7210, International Rectifier) to provide current to each motor. The shift register of each control card is connected serially to that of the adjacent cards via the serial connection, stores the positions of all the motors, and provides the control signals for the timing of the stepping motors. Software for the microcontroller was written in C, converted to assembly code and downloaded using the software package AVR Studio 4 (Atmel). The serial communication protocol utilizes ASCII command strings, which are newline character terminated. Error detection is accomplished using an odd-parity bit. The high level serial (RS-232) signals are level shifted to the I/O voltages required by the microcontroller using a MAX232 interface chip.

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backplane card. This allows the microcontroller to select a specific motor on any of the eight control cards.

Communication between the EMC and the RMIU is routed through an eight conductor cable carrying power, coil signals, and motor selection data. To select a specific motor, the microcontroller shifts a data word, containing a single high bit, into the shift registers on the motor control cards in the RMIU. When the shift register output is latched, the selected N-channel MOSFET connects the common coil wire to ground on the stepping motor. All other motors are kept inactive because their connection to ground is held at high-impedance. The microcontroller then switches the P-channel MOSFETs to connect the motor coils to +5 V in sequence to move the motor (see Fig. 10).

Each manipulator is connected to the RMIU by a 32-conductor flat flex cable (Parlex). The connectors for these cables (52559×3292, Molex) are mounted on the back side of the RMIU card (Fig. 1D, supplementary material).

Software

The graphical user interface (GUI) was written in the C language using the National Instruments GUI libraries for user control, runs on the Windows operating system, and communicates with the EMC by serial/RS-232 protocol. Figure 2 in the supplementary material shows a screen shot of the GUI that provides user input to control the micromanipulator. The user can set the motor speed, control the direction and the number of steps for each movement command, and set the movement resolution of the device according to the number of threads/inch on the lead screws. Once a motor is selected, the position reading can be zeroed, and each electrode advanced independently. At the end of a session, all of the electrodes can be returned to their zeroed positions using a GUI command.

Microdrive parameters

The operating parameters of the microdrive system are given in Table 1 and are based on measurements taken from seven fully assembled systems.

RESULTS

Chamber system and dural removal

During the past 3 years, we have implanted chambers, and performed subsequent electrophysiological recordings, at 11 locations in five monkeys. At all but three locations, the chambers were positioned over area V1 near the occipital pole. In one animal, a chamber was implanted over area V4, and in another animal, we implanted two chambers at the same time, one over the intraparietal sulcus, and a second over the principal sulcus. Although it is not necessary to remove or thin the dura to use this chamber system, we tested the performance of the chamber under such conditions. At five of the chamber sites, we completely removed the dural membrane overlying the cortex. This was done after the chamber was implanted by carefully lifting the dura using a pair of fine forceps and incising the membrane around the inner perimeter of the chamber using a No. 11 scalpel. At the remaining six locations, we thinned the dura prior to recording by carefully peeling away several layers of the membrane until the underlying cortical vasculature was just visible. In all instances, we performed these procedures using sterile technique, and the tissue was flushed using artificial cerebrospinal fluid (ACSF) at physiological pH (7.2–7.4) and temperature (38°C) (Arieli et al. 2002). The use of ACSF greatly reduced the amount of bleeding compared with similar procedures done with physiological saline. To further reduce the inflammatory response of the dura, the animals were given dexamethazone (im) during and after the surgery at 24-h intervals for 5 days (0.5 mg/kg).

We typically began neuronal recording 2–3 days after removing or thinning the dura. At those sites where the dural membrane was removed, we observed two types of tissue responses that were visible through the Sylastic membrane using an operating microscope. In two cases, the cortical surface was in direct contact with the Sylastic membrane, presumably due to positive intracranial pressure. This was the response we had hoped for when designing the chamber system, and it allowed accurate recordings of neuronal activity

<table>
<thead>
<tr>
<th>Electrode Travel, mm</th>
<th>Travel Resolution, μm/step</th>
<th>Maximum Velocity, μm/s</th>
<th>Positional Lag, μm</th>
</tr>
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<tbody>
<tr>
<td>11</td>
<td>0.66 μm/step</td>
<td>168</td>
<td>70–100</td>
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to be made from the upper layers of the cortex. In the other three cases, the cortex appeared to lie 1–2 mm below the Sylastic membrane, with clear CSP filling the space. In all five cases, we observed a gradual invasion of granular tissue over the surface of the cortex that eventually led to the replacement of the dural membrane. This process varied in rate from one chamber site to another, and typically was complete by 6–8 wk after the initial dural removal. In two cases, we attempted to prevent the growth of this membrane by first removing the sleeve and then removing the connective tissue with forceps. This approach was largely ineffective because regrowth was rapid and in one instance we damaged some of the small pial vessels.

Overall, our approach to the problem of dural regrowth differed substantially from that of Arieli et al. (2002). In their study, it was essential that the dural membrane be prevented from re-growing, otherwise optical imaging from the cortical surface would become impossible. Our objective in removing the dura was to facilitate the entry of multiple electrodes into the surface cortex and avoid the substantial dimpling that occurs in these procedures when the dura is intact. We therefore made no attempt to block the re-growth of the dura and did not carefully track the time course or properties of the dural regrowth.

Once the regrowth of the dural membrane was complete, we made several attempts to remove the newly formed dura. In general, we found this to be difficult, as it appeared to us that the dura had become integrated with the pial surface during its regeneration. Attempts to remove it led to slight damage of the pial vessels in two instances. Overall, we found that the best approach was to leave the original sleeve in place and avoid any manipulations of the tissue for as long as possible. When we took this approach, we could routinely make electrode penetrations into the surface cortex without any detectable dimpling for periods lasting 4–6 wk. Once the regrown dura had thinned, thinning procedures could be done with little or no difficulty.

At the six chamber sites where the dura was thinned, but not completely removed, electrodes would readily penetrate the dura for a period of 2–4 wk. After this period, it became necessary to repeat the dural thinning procedure. We repeated this procedure as many as six times allowing for 3–6 mo of reliable recording. At these sites, the interior of the recording chamber remained free of infection.

**Electrophysiological recording**

We have performed extensive multielectrode recordings of neuronal activity using three of the microdrive systems. In general, the system has performed well (problems are discussed in the following text) and high quality broadband (1 Hz to 10 kHz) recordings, having low noise, were readily obtained on a daily basis. An example of raw data collected on one such recording is shown in Fig. 11. In this experiment, four electrodes were advanced into area 7a of the posterior parietal cortex. A broadband signal was sampled from each electrode, enabling subsequent separation of the local field potential signal (1–150 Hz) and a high-pass signal containing unit activity (500 Hz to 10 kHz). Single-unit activity was extracted off-line from the high-pass signal using a semi-automated spike sorting algorithm (Goodell and Gray 2003; Yen et al. 2006).

On a typical recording session, prior to mounting the manipulator on the animal, and after sterilization of the electrode tips, we positioned each electrode so that its tip was just visible at the distal opening of the guide hole. The electrode position was set to zero using the GUI. Each electrode was then retracted 0.5 mm to avoid damage to the tip during mounting. The microdrive was mounted on the chamber and the electrodes were advanced sequentially in increments of 100 steps (67 μm). When an electrode passed through the Sylastic membrane, a clear transition from open-circuit noise to a detectable field potential could be observed. This typically required 100–300 μm of movement from the zero position. We usually advanced all of the electrodes to this point, when recording from surface cortex, before beginning the search for neuronal activity. This helped to minimize subsequent mechanical disturbances because the dimpling of the membrane occurred before any neuronal activity was isolated. The electrodes were then sequentially advanced in 100-step increments until multunit activity could be detected on at least one electrode. At this point, electrode movement was reduced to 10- or 20-step increments. Subsequent movements were then used to maximize the signal-to-noise ratio of the unit activity.

Several aspects of this recording sequence are noteworthy. First, well-isolated single units were not always recorded simultaneously on every electrode. This is evident in Fig. 11. Attempts to improve the isolation on one electrode often influenced the isolation of units on adjacent electrodes. Because of this, we found that the best way to maximize unit isolation across electrodes was to first obtain multunit activity on all the electrodes, let the recording stabilize for 10–30 min (or longer) and then make small gradual changes in electrode position (1–10 μm) to improve signal quality. When well-isolated units were obtained, it was often helpful to retract the electrode to improve or maintain the isolation. Second, because of the large currents needed to activate the stepping motors, it was not possible to monitor neuronal activity during electrode movement. This usually was not a problem because small movements were completed in less than a second. Third, because the microdrive was designed to act as its own Faraday...
cage, interference from 60-Hz line noise was often undetectable. This enabled recording of the broadband signal without the need for additional shielding or filtering. Finally, recording stability was typically very good. Isolated units could routinely be held for periods lasting 1–2 h. The principal factor affecting recording stability was the presence of an air-tight seal within the chamber. Recording stability was compromised whenever an air bubble was present below the surface of the Sylastic membrane prior to recording. This often led to detectable cardiac pulsations in the broadband signal and consequent variations in signal quality.

### Sylastic membrane

The Sylastic membrane appeared to have very little effect on the recording properties of our electrodes. After repeated recordings using a variety of electrode parameters, we settled on the use of Tungsten-in-glass electrodes manufactured by Alpha Omega (125 μm wire, 300 μm OD, 1 MΩ impedance, 60° taper angle). Electrodes of smaller diameters, or finer tip tapers, occasionally had bent tips or reduced impedance after penetrating the Sylastic membrane. With the current electrode parameters, we routinely used the same electrodes three to six times and usually found little or no change in the electrode impedance, when tested at 1 kHz. We and others have also successfully used other types of electrodes, including epoxy coated Tungsten electrodes manufactured by FHC (M. Leslie, Vanderbilt University, personal communication).

The Sylastic membrane also showed little resistance to electrode penetration and, in most cases, was self-sealing. Evidence for this was apparent in those instances where the dural membrane had been completely removed. In some of these recordings, we encountered neuronal activity within 400 μm of the starting position of the electrode within the guide tube. This indicates that very little dimpling of the membrane occurred.

There was often a small droplet of residual fluid present on the surface of the membrane once the electrodes had been withdrawn. This suggested that the membrane had either not completely sealed around the electrode during a recording or had not resealed after the electrode was withdrawn. The amount of residual fluid was always very small, and we found no evidence that fluid from the cranial cavity had wicked up into the holes in the guide array. This suggests that the residual fluid had escaped during withdrawal of the electrodes. In these cases, we usually removed the fluid with gentle suction, using a sterile pipette, before sealing the chamber and returning the animal to its cage. This manipulation appeared to facilitate the resealing of the membrane as we often found the chamber to be completely dry the following day. This effect was not repeatable indefinitely, however. The membrane typically began to leak after 5–10 electrode penetrations at the same site. When this occurred small amounts of fluid would accumulate in the chamber.

When electrophysiological recordings were not being performed, the Sylastic membrane maintained a stable water-tight seal for many months. In one animal, the membrane was left in place for 18 mo before the dura was thinned, and recordings were begun. At the time of thinning, the dural membrane was healthy and appeared only marginally thicker than it was at the time the craniotomy was performed.

In general, infections have been either rare or nonexistent. This has been one of the big benefits of the technique because maintaining a sterile barrier eliminates the need for daily cleaning of the chamber and reduces the rate of regrowth of the dural membrane. When infections have occurred, they have been very difficult to eliminate as is often the case with conventional recording chambers. In these instances, we have found it necessary to remove the sleeve and replace it with a sterile sleeve two to three times each week. This procedure can be done in 10–15 min, without anesthetizing the monkey, provided aseptic technique is used.

### Using multiple microdrives

One important advantage of the system is that it permits the simultaneous use of multiple microdrives, enabling the sampling of neural activity from multiple brain areas. This is possible because of the small dimensions of the chamber, the relatively small size and light weight of the microdrive, and the capability of the electronics and software to operate ≥64 motors at a time. We made use of this capability in one monkey by implanting two recording chambers, one over the intraparietal sulcus, targeting areas 7a and LIP, and one over the principal sulcus, targeting areas 9 and 46 of the prefrontal cortex. In general, the addition of a second chamber posed no significant mechanical obstacles other than increasing the time needed to prepare the microdrives and advance the electrodes during each recording session. Electrically, however, the addition of a second microdrive presented at least two difficulties. First, because the microdrive is designed so that the ground connection passes through the recording chamber, the addition of a second chamber introduced the possibility of creating a ground-loop, and hence significant line noise. Second, because each drive is fitted with an 8-channel connector, it became necessary to use either two 8-channel amplifiers or to build an adapter that would connect all 16 channels to a single 16-channel headstage. We solved the ground loop problem by using only a single ground and passing all of the signals to a single main stage amplifier.

### DISCUSSION

We have designed, built, and extensively tested a new type of chamber and microdrive system for performing acute multielectrode recordings of neuronal activity in awake behaving monkeys. The system offers a number of advantages over existing technology. The chamber is small, is low in profile and incorporates a replaceable sleeve containing a thin Sylastic membrane that is easily penetrated by microelectrodes. The small size minimizes the amount of cranial surface area taken up by the chamber and facilitates the implantation of multiple chambers on a single animal. The low profile permits easy surgical access to the chamber interior, which facilitates procedures such as dural thinning or removal. The replaceable sleeve allows a hermetic seal to be established. This greatly reduces the susceptibility to infection, eliminates the need for daily cleaning, and reduces the rate of dural thickening that occurs in the absence of a seal. The seal also permits complete removal of the dural membrane, a manipulation that would otherwise be very risky with conventional chambers. The Sylastic membrane is very thin, can be easily penetrated with
consistent difficulty, which showed up after prolonged use of the system. First, the actuators software changes. Mechanically, we discovered three problems of the system. These included mechanical, electrical, and of useful design changes became apparent after extensive use mounting the Sylastic membrane onto the sleeve.

Finally, it would be useful to construct the chamber out of vertically within the chamber, and it would not be possible to over, the internal sleeve would have to be redesigned to mount the top of the guide array. We solved this problem by incorporating a set of eight stainless steel guide tubes that could be press-fit into selected holes in the top of the guide array prior to recording, and held in alignment with each actuator using a grid mounted on the interior of the support arms (the grid is visible in the middle of the microdrive in Fig. 4A). Finally, we also found that it was easy to mistakenly retract or advance the actuators beyond their upper and lower positional limits. When this happened, there were times when the resulting friction would cause the plunger to stick in place, and these instances were not registered by the software and were thus not detected by the user. Short of incorporating a separate position encoder for each actuator, which we ruled out due to size, complexity and expense, the best solution is to incorporate end limits within the software which can be set at the beginning of each recording session.

Electrically, we found very few problems overall. The only consistent difficulty, which showed up after prolonged use of the system, was the presence of occasional intermittent signal connections between the BeCu tabs on the PCB and the BeCu strips on the plungers. We discovered that these problems were due either to the build up of an oxidation layer on the surface of the BeCu contacts or a slight bending of the tabs making the connection between strips and tabs unreliable. We solved the first problem by occasionally cleaning the surfaces of the contacts. The latter problem was solved by redesigning the BeCu tabs so that they were thicker and longer. This increased the force of the spring loaded contact between the tabs and the strips on the plungers.

Although we have mentioned several changes to the software that improved the design or eliminated problems, we also found that the system could be greatly improved by including software modifications to permit automated electrode movement sequences. This was particularly evident when advancing multiple electrodes each day, which often required a great deal of time by the user. This capability should be relatively easy to implement and would enable the user to specify a wide variety of movement sequences.

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