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Ultra-miniature headstage with 6-channel drive and vacuum-assisted micro-wire implantation for chronic recording from the neocortex

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Abstract

We describe a head-stage, with precision microtranslators for the chronic placement of micro-wire electrodes in the neocortex, that minimizes compressive damage to the brain. The head-stage has a diameter of 5.8 mm and allows six electrodes, separated by 450 μm on a hexagonal grid, to be individually and continuously positioned throughout a depth of approximately 3 mm. Suction is used to transiently support the dura against a curved array of tubes that guide and stabilize the electrodes as a means to prevent compression of the neocortex as the electrodes breach the dura. With this headstage we recorded extracellular signals in a rat immediately after surgery. Single-unit waveforms at a given electrode position were stable for at least several hours in the freely behaving animal and were obtained throughout the depth of the neocortex for at least 2 months. Electrophysiological records and histological examination showed that the upper layers of the neocortex were intact and minimally damaged after the implantation. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Extracellular recording with electrodes formed from fine wire is the singular method used to record the electrical activity of neurons in freely moving animals (Strumwasser, 1957) (for reviews see Kruger (1983) and Lemon (1984)). The major experimental focus has been on primates and rodents, both of which can be trained to perform tasks concurrent with electrophysiological recording. For the case of primates, an indwelling fixture is chronically implanted on the head of the animal to allow access to the dura. These measurements largely involve animals constrained to sit in a chair with their head immobilized, but their bodies otherwise free to move, so that an array of electrodes which are attached to the head fixture may be lowered into the brain and removed on a daily basis (Humphrey 1970; Reitbock et al., 1981). For the case of rodents, head-immobilized preparations are a new development that should prove valuable in the study of vibrissa movement (Bermejo et al., 1996). However, much of the advantage of the use of rodents lies in the ability to study neuronal correlates of behavior involved in locomotion and search (O'Keefe and Dostrovsky 1971; Ranck 1973). In this case, the entire recording apparatus must be fixed to the head of the animal and must not be intrusive to permit the free movement of the head and limbs.

The pioneering experiments of Chapin and Woodward (Chapin and Woodward, 1982a,b) demonstrated the feasibility of recording single-unit activity from the neocortex in the rat during behavior and paved the way for more recent work on the relation between electrical activity in the neocortex and sensory-motor tasks (Taube et al., 1990; Chen et al., 1994; McNaughton et al., 1994; Nicolelis et al., 1995; Carvell et al., 1996; Fee et al., 1997). Nonetheless, several difficulties still plague the recording of single-unit activity in the neocortex of

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free ranging animals. First, there is a need for stable, precise and independent positioning of several electrodes within a particular area of the cortex, as has been accomplished for the case of the hippocampus (Gothard et al., 1996; Wilson and McNaughton, 1993). Individual electrodes must be adjusted on a regular basis to insure their continued sensitivity to single-units, as well as to enable different layers of the cortex to be sampled within the same animal. Secondly, the headstage must be small enough so that multiple devices can be used to simultaneously probe different brain areas. Thirdly, the device must be sufficiently small and light in weight so as not to perturb the animal; this will become more critical as the use of transgenic mice becomes prevalent (McHugh et al., 1996).

A final issue concerns the need to implant electrodes without the need to cut the overlying dura, which can cause bleeding and may expose the cortex to edema and infection, yet without damaging the neocortex through the compression of the upper layers as the electrodes first impinge on the dura. Single, sharp microelectrodes may penetrate the dura rather easily if it is tensioned as the electrode advances; this is achieved when the penetration is made through a small (<1 mm diameter) craniotomy. However, the sequential or simultaneous implantation of multiple micro-wire electrodes into the neocortex through a larger craniotomy, necessary for multiple recordings within one area, is likely to produce a significant compression of the neocortex leading to the subsequent depression of single-unit activity.

Here we describe a multi-electrode headstage for chronic recording with rodents, whose design and realization were motivated by the above issues. A captured screw and threaded shuttle form each of six parallel axes. The implantation of the electrodes exploits a vacuum technique that alleviates compression of the dura. The fabrication of the device exploits standard components and involves conventional, albeit high precision, metal machining techniques. Our realization uses two-wire microelectrodes, i.e. stereotrodes (McNaughton et al., 1983), but the device readily accommodates four-wire microelectrodes, i.e. tetrodes (O'Keefe and Recce, 1993; Wilson and McNaughton, 1993; Gray et al., 1995).

2. Methods

Our subjects were female Long–Evans rats, 250–300 g. Issues related to the training of animals and surgical procedures have been described elsewhere (Fee et al., 1996a, 1997). We record regular and fast spiking units (Simons, 1978) from layers 2–6 of the vibrissa area of the primary somatosensory cortex and primary motor cortex. The care and experimental manipulation of our animals are in strict accord with the guidelines from the National Institutes of Health (1985) and have been reviewed and approved by the local Institutional Animal Care and Use Committee.

At the end point of the experiments the animals were sacrificed and perfused as previously described (Kleinfeld and Delaney, 1996). To minimize disturbance of the cortical tissue in the vicinity of the electrodes, the electrodes were retracted, and the microtranslator removed, following perfusion. The brains were stored at 4°C in 4% paraformaldehyde in 100 mM phosphate buffer for further processing.

2.1. Headstage design

The device consists of three chambers that stack on each other, along with a lid, internal parts, electrical connectors, and a cap for protection (Fig. 1). The upper and middle chambers and the lid form a self-supporting unit of electrode holders and translators. The lower chamber is anchored to the skull of the animal and provides a seat for the above unit.

A set of six shuttles are supported by slots in the upper chamber and are driven by lead screws. The lid and the middle chamber capture the screws, so that rotation of a lead screw leads to translation of the shuttle and a change in penetration depth of the electrode (Fig. 1). We used 160 pitch screws; this resulted in a travel of 159 μm/turn. The maximum travel of the shuttles is constrained only by the height of the upper chamber.

The lateral position of the electrodes is constrained by a hexagonal array of guide-tubes that is held by the middle chamber and extends through the lower chamber to a level just above the dura (Fig. 2b). The bottom lip the array further acts to support the dura during implantation of the electrodes and is shaped to approximately match the curvature of the brain. The bottom of the lower chamber matches this curvature. The electrodes are held in one of two radial positions by the shuttle, corresponding to either the inner or outer circle of the array.

The machine plans for each of the major parts are given in Appendix A (Fig. 5), along with construction details for the guide-tube array (Fig. 6). The major parts, with the quantities in parenthesis, are the lid (1), the upper chamber (1), the shuttles (6), the middle chamber (1), the guide-tube array (1), and the lower chamber (1). In addition there are spacers (3) between the lid and upper chamber. All parts are fabricated with conventional and electric discharge machining from #304 stainless steel. All screws are size 0000 with slotted oval fillister heads and made of #303 stainless steel (J.I. Morris, Southbridge, MA). Those that hold the lid to the upper chamber (3), the guide-

tube array to the middle chamber (2), and the upper chamber to the middle chamber (3) are 1/16 in.; and those that secure the upper and middle chambers to the lower chamber (3) are 1/4 in. The lead screws (6) are formed from size threaded rod, as described (Appendix A). All metal tubing is # 304 stainless steel hypodermic tubing (Small Parts, Miami Lakes, FL) and was cut with a 180 grit abrasive wheel in a cut-off saw (CUT-4, 0-CW-4 and 0-CD-1; Small Parts).

2.2. Stereotrodes

The dual-wire electrodes, or stereotrodes, used in our experiments are similar to those previously described ((McNaughton et al. (1983) following Verzeano (1956)) (Fig. 2b). The stereotrodes are formed from a twisted pair of 25 μm parylene-coated tungsten wires (size 0.001 tungsten 99.95% H-ML; California Fine Wire, Grover Beach, CA), with a beveled and gold plated electrode edge, as detailed in Appendix B. Each stereotrode is held in a 31-gauge tube (Q-HTX-31; Small Parts), 0.56 in. in length, that is attached to a shuttle and slides along a guide-tube (Fig. 2b).

2.3. Assembly

Each of the six lead screws and shuttles is assembled within the upper chamber (Fig. 2a and b). Next, the guide tube array is placed in the middle chamber and aligned relative to the upper chamber so as to allow the electrode support tubes to be inserted through each shuttles and into the corresponding location in the guide-tube array. The height of the guide tube array is adjusted (typically so that it protrudes approximately 1 mm beyond the lower chamber), and then the array is fastened to the middle chamber by the lock screws and the middle chamber is attached to the upper chamber with 1/16 in. screws (Fig. 2c). The partially completed headstage is now ready to receive the electrodes.

The electrode support tubes are inserted so that a 0.200 in. length protrudes above the shuttle, and are cemented in place (Superbonder 49550; Loctite, Hartford, CN). The stereotrodes are inserted into each of the support tubes such that their tips lie just flush with the bottom of the guide-tube-array, and then are cemented to the top of their support tube. This arrangement permits up to a 0.120 in. length of electrode to

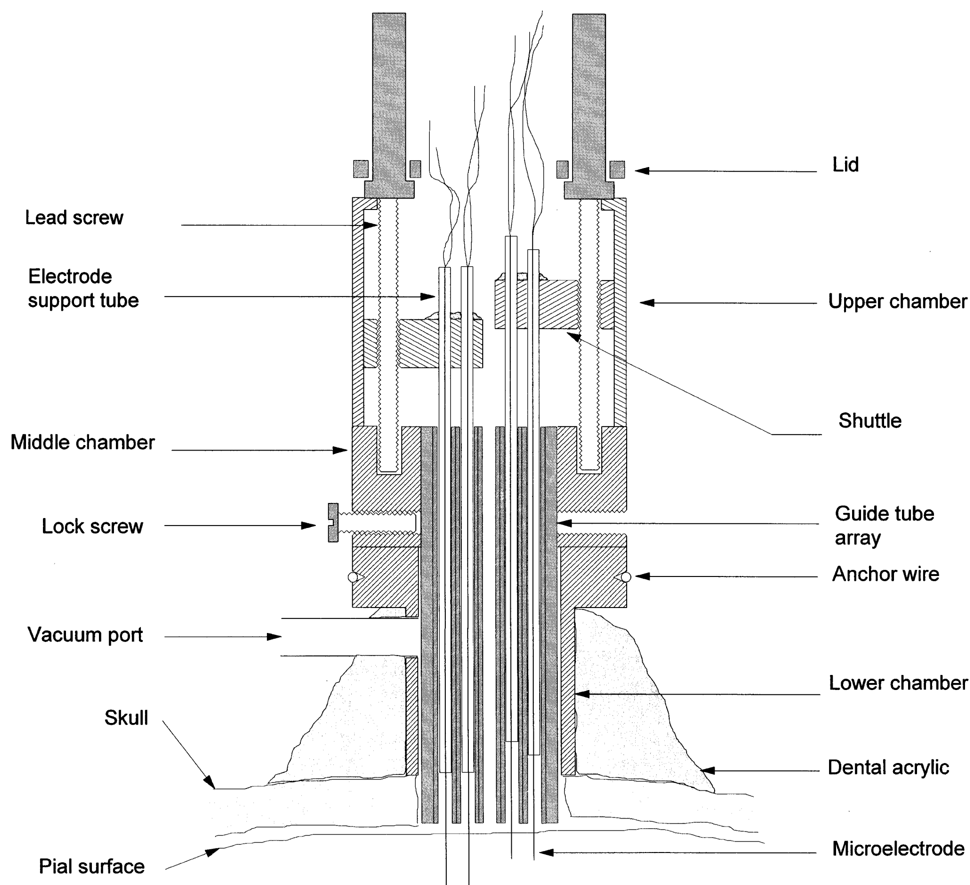

Fig. 1. Schematic overview of the headstage. A lead screw controls the height of the shuttle, which in turn controls the height of the electrode. The vacuum port is used only at the time that the stage is mounted and each of the electrodes is lowered to breech the dura. Note that the electrodes are shown to occupy both radial positions solely for purposes of illustration. In practice, a given shuttle will contain only one electrode.

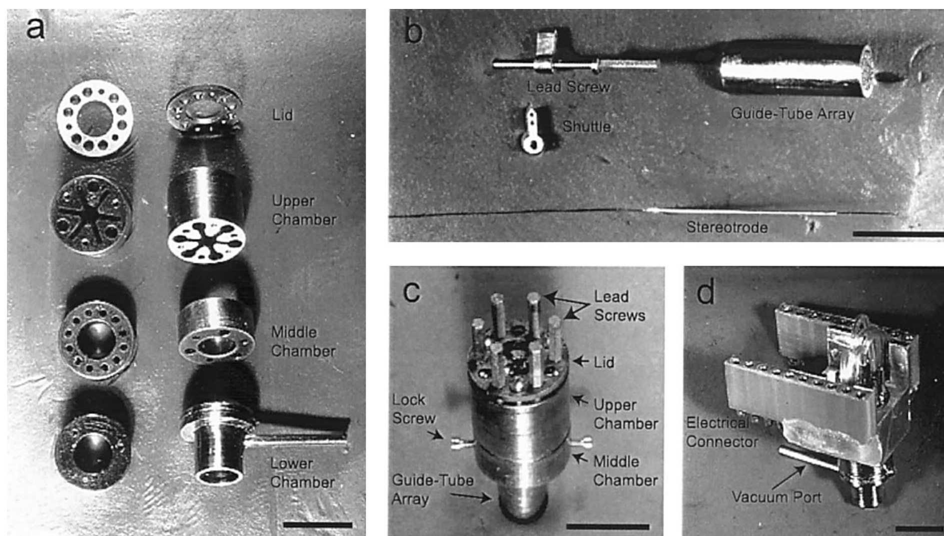

Fig. 2. Photographs of the components of the headstage at various levels of assembly. (a) The lid and all three chambers are shown from angled perspectives from the bottom and from the top. The tube in the lower chamber is the vacuum port. (b) The lead screw and shuttle, shown separately and assembled, along with the guide-tube array and a stereotrode in a support tube. (c) Top view of the partially assembled headstage consisting of the shuttles and lead screws, along with the lid, upper and middle chambers, the guide-tube array, and the lock screws that secure the array. (d) The assembled headstage, including electrical connectors and an aluminum fixture to hold the connectors and protect the stereotrode leads. Note that only six of the 10 pin-outs are connected and potted with dental acrylic; the remaining pins are used for reference and, in our experiments, electromyograph wires. The scale bar in all panels is 5 mm.

protrude as the shuttle is lowered. Lastly, the lid and spacers are attached with 1/16 in. screws (Fig. 2a and c).

Each pair of stereotrode leads is sheathed in a short segment of silicone tubing (8106; A-M Systems, Everette, WA). Connection to the tungsten electrode wires is made by soldering (Stay Clean Flux; Harris, Cincinnati, OH) to two, 10-pin, 0.050 in. pitch dual-row strips (we also use the SFMC series connectors from Samtec (New Albany, IN), which have a 0.0315 in. pitch that are cemented (Torr Seal; Varian Vacuum Products, Lexington, MA) to a fixture that is fastened to the upper chamber (Fig. 2d). The soldered connections are potted with dental acrylic. A detachable signal-buffer contains a matching connector along with common source field effect transistor amplifiers (HD18 or equivalent; NB Laboratories, Denison, TX).

The buffered signals are transmitted along a 1 m cable together with a buffered cortical reference signal, and amplified with a gain of 100 by an instrumentation amplifier (INA101; Burr-Brown, Tucson, AZ) immediately before they pass through a commutator (IDEA Development, Anaheim, CA) and are further amplified, filtered, and digitized.

2.4. Electrode implantation

We focused on the use of the microtranslator to record from the primary somatosensory cortex in rat. With the animal under anesthesia, a 3 mm circular craniotomy, when viewed from above, is made over the parietal cortex. The intact dura is carefully cleared of bone. A series of 00–90 threaded holes are tapped and 00–90 filister head screws are placed in the top of the skull to act as anchors for the apparatus and are tied together with 0.003 in. stainless steel wire; the screws also serve as the animal ground. A second, approximately 500 μm circular craniotomy is prepared outside the location of the lower chamber. A 0.003 in. TFE coated tungsten wire (no. 7955; A-M Systems) with the bottom approximately 500 μm cleared of insulation and with the tip sharpened as above, is slowly positioned into the neocortex to serve as a reference. This wire is anchored with dental acrylic.

The lower chamber is centered over the exposed dura and a final check is made to ensure that the outer edges of this chamber make close contact with the bone. The anchor and reference wires are then encapsulated in dental acrylic. The extent of protrusion of the guide-tube array is checked to ensure that it matches the depth of the hole above the dura. Small corrections to the position of the guide-tube array may still be made at this point. The array is inserted into the lower chamber and the middle and lower chambers are screwed together (Fig. 2d).

A vacuum of approximately 15 in. of mercury is applied to the port on the side of the lower chamber (Fig. 1 and Fig. 2a and d). Under the application of the vacuum, the dura is pressed against the curved bottom surface of the array by the pressure of the cerebrospinal fluid. The electrodes are advanced slowly (approximately 100 μm/min), one at a time, until a dramatic rise

in electrical activity is heard on an audio monitor to indicate that the electrode has breached the dura. When all the electrodes have penetrated the dura, the vacuum is released. The animal is allowed to recover from the anesthesia and returned to its cage. After a day or two, the electrodes are advanced slowly while the animal is awake, until neuronal signals are seen.

3. Results

The final device is 5.85 mm in diameter and has a mass of 1.4 g, independent of the electrical connections, and 2.7–3.3 g with two 10-pin connectors and a support fixture (the smaller mass refers to the use of two 7-pin connectors and a polycarbonate fixture to secure the connectors; the larger mass refers to the use of two 10-pin connectors and a (reuseable) aluminum fixture) (Fig. 2d). Adjustment of the electrodes is made by cradling the animal and turning each lead screw while the electrical signal is displayed or broadcast on an audio monitor.

3.1. Recording

Stable recordings are obtained immediately after surgery. Single-unit recordings are stable for at least several hours in the freely behaving animal. An example of sorted waveforms (Fee et al., 1996b) from one such record obtained from a stereotrode in layer 2/3 is shown in Fig. 3. Such records are largely impossible to obtain if the surface of the cortex is compressed. Useful single-unit signals were obtained for the 1–2 month periods of our experiments (Fee et al., 1997).

3.2. Histology

Postmortem examination of the brains of five animals in which recordings of neocortical electrical activity were obtained indicated no signs of gross morphological disturbance of the cortical surface. This is consistent with the absence of compression of the neocortex as the electrodes breech the dura. A more comprehensive assay for tissue damage was achieved by preparing thin sections of the brain tissue in the vicinity of the electrode tracks and immunoreacting these sections with antiserum for vimentin, a filament protein in astrocytes, as described by Friedman et al. (1989). The expression of vimentin is normally absent in adult gray mater and sparse in white matter (Friedman et al., 1989), but is strong in reactive astrocytes that proliferate in response to injury (Latov et al., 1979).

We prepared 30 μm serial sections of tissue in the vicinity of the electrode tracts. We observed that the density of vimentin-positive cells ranged from negligible in most sections to a worst case in which the path of a stereotrode was defined by vimentin-positive cells (* in Fig. 4a). High magnification views show that the vimentin positive cells are clearly astrocytes (Fig. 4b and c). The density of reactivity is significantly greater than that in the contralateral hemisphere, but similar to that observed in white matter (Fig. 4a).

4. Discussion

The first electrode head-stage for the study of neuronal activity in free ranging rodents was described by Strumwasser (1957). The current device builds on this and subsequent designs (Ranck, 1973; McNaughton et al., 1983; Kubie, 1984; Gothard et al., 1996), particularly the single electrode lead-screw design of Wall et al. (1967). Our headstage allows up to six multi-wire electrodes to be implanted into a 0.6 mm^2 region of cortex and allows the experimenter to precisely change the position of each multi-wire electrode on a regular basis by adjusting a microtranslator. The operation is based on a captured screw and a threaded shuttle that carries the electrode. The ability to control the level of the individual electrodes assures that each wire can reliably detect single-unit signals. The small diameter and weight of the device should allow multiple units to be placed on the head of an animal so that many regions of the neocortex may be probed at the same time.

As part of our design we introduced a vacuum assisted implantation technique that avoids compression of the brain as the electrodes first breech the dura. Compression of the brain has been a major difficulty

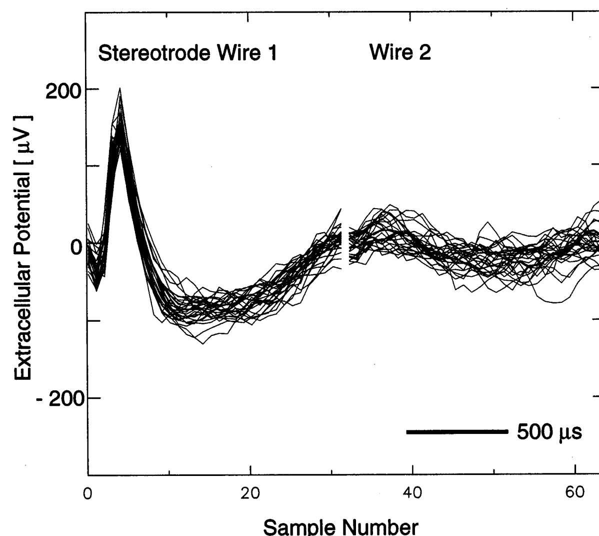

Fig. 3. Sorted stereotrode spike waveforms from a depth of approximately 250 μm below the pial surface. Shown are 30 randomly selected examples from 150 waveforms for this unit. The time per sample is 0.40 ms. The waveforms from the two electrodes are shown as a continuous 64 element vector, as used in our spike sorting algorithm (Fee et al., 1996b).

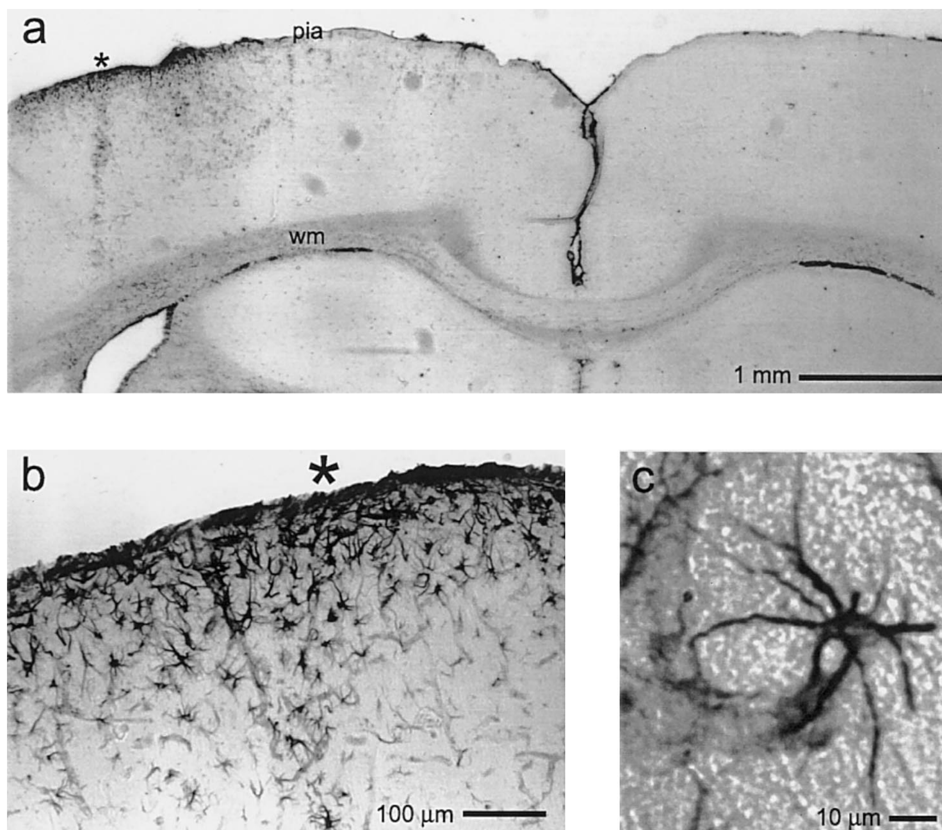

Fig. 4. Photomicrographs of a 30 μm section, at the level of one stereotrode, immunostained for vimentin. (a) Low magnification brightfield view (pia, pia mater; wm, white matter). The * denotes the approximate location of the stereotrode tract; note the presence of immunostaining. The staining observed medial to the tract is the result of damage from lowering a reference electrode approximately 2 mm from this tract. (b) Intermediate magnification brightfield view at the position of * in (a). (c) High magnification view, with Normarski optics, to illustrate that the positively stained cells are astrocytes.

and leads to the depression of local brain activity for hours or days. As such, many investigators use a lateral approach to avoid the risk of damage to the area of interest. With the present and related designs, we have recorded from the somatosensory cortex in multiple animals (Fee et al., 1996a, 1997) as well as from the motor cortex (Venkatachalam et al., 1998).

The translators in the present design are fabricated near the practical limits of machining capability. With the electrodes arranged at the inner circle in the guide-tube, the aerial density of the electrodes is approximately 1.5% of the cortical area. This density is presently limited by mechanical constraints on the support of the electrodes by shuttles. While finer screws than those used in this project have been used by watchmakers (the unified miniature screw thread (UNM) standard specifies a 0.30 UNM screw, which has a diameter of 0.0118 in. and a pitch of 318 threads per inch), we suspect that a substantial increase in the density of individually adjustable electrodes will have to await the fabrication of appropriately micromachined silicon actuators.

5. Note in proof

The dimensions shown in Fig. 5b and 5c for clearance of the filister screw heads should be 0.036 in., and not 0.035 in. as indicated.

Fig. 5. Mechanical drawings of components of the headstage. (a) Lid: 'A' is a through hole of 0.035 in. in diameter, 'B' is a through hole 0.033 in. in diameter, and 'C' is a through hole to pass a 0000-160 screw (0.021 in. diameter). (b) Upper chamber: 'A' is a through hole counterbored to pass a 0000 filister head screw (0.021 in. diameter), 'B' is a through shaft for the shuttle, and 'C' is a through hole threaded 0000-160 on both ends. (c) Middle chamber: 'A' is a through hole to pass a 0000-160 screw (0.021 in. diameter), B' is a bottom-out hole to accept the 0000-160 lead screw, and 'C' is a through hole counterbored to pass a 0000 filister head screw (0.021 in. diameter). (d) Lower chamber: 'A' is a threaded through hole (0000-160). This bottom of this part is modified for the curvature of the underlying bone; in the upper example it is matched to the vibrissa motor cortex and in the lower example it is matched to the primary vibrissa somatosensory cortex. (e) Lead screw head. (f) Shuttle. All tolerances are ± 0.0005 in. unless four places of precision are specified, in which case the tolerances are ± 0.0002 in.

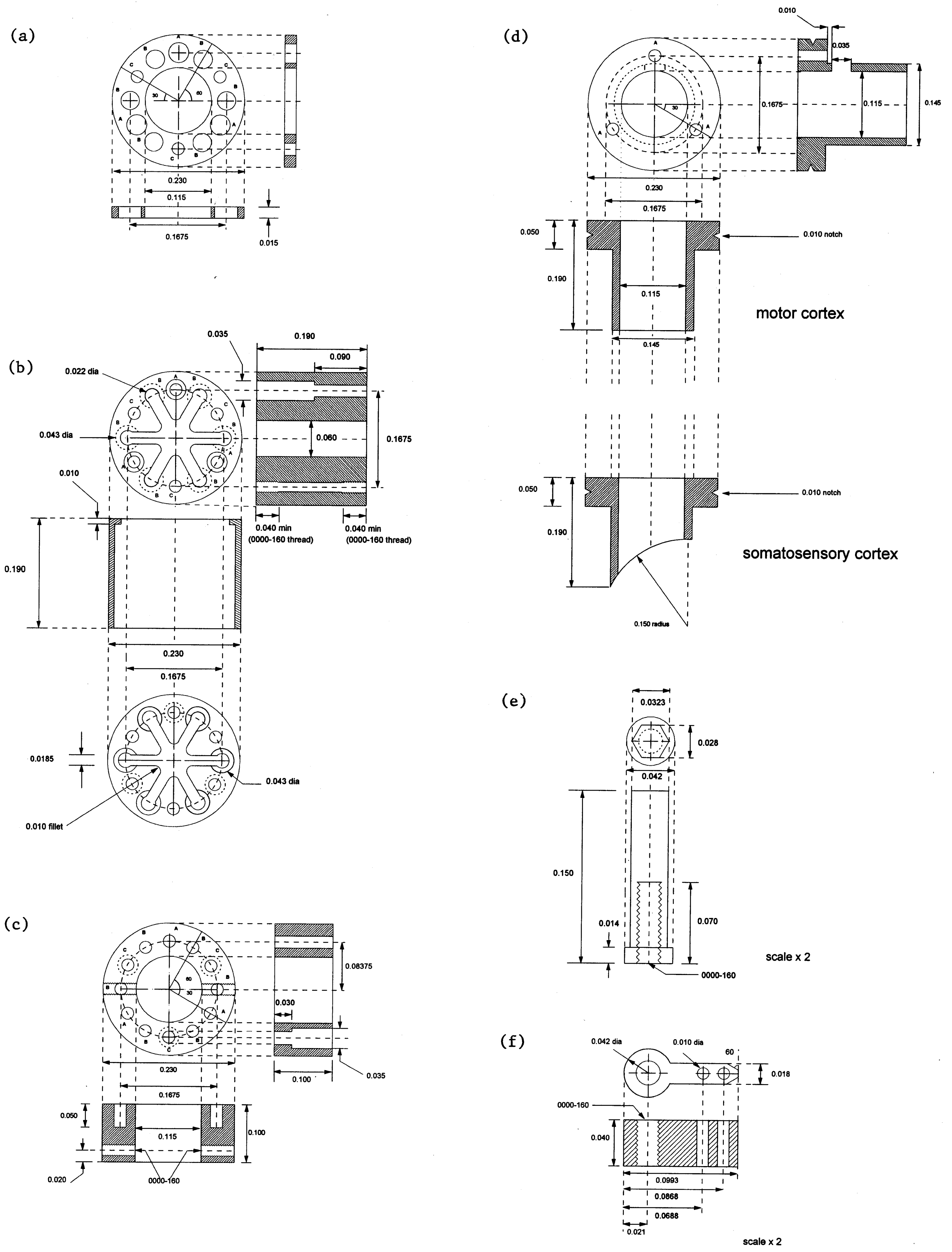

Fig. 5.

Acknowledgements

The design of the present headstage was an interactive process, such that SV and MSF should both be considered as 'first' authors. We thank P. Boland and B. Friedman for performing the histology, H.J. Karten for use of his photomicroscope, J.K. Chapin, B.L. McNaughton, M.A.L. Nicolelis, and M.A. Wilson for discussions on their extracellular techniques and A. Khabbaz for comments on the manuscript. Fabrication was carried out at Advanced Machine and Tooling, Inc., San Diego, CA (www.amtmfg.com). This study was supported by the Whitehall Foundation, the Buroughs-Wellcome Foundation, and by Lucent Technologies.

Appendix A. Headstage fabrication and machinist drawings

Lid (Fig. 2a and Fig. 5a): This part has 12 holes that are uniformly divided on a fixed radius. Six alternately spaced holes act to capture the lead screws and permit access for turning the screw. Three of the remaining holes are sized to pass size 0000 screws so that the lid can be screwed to the upper chamber. The final three holes provide access to counterbored screw holes in the upper chamber that anchor the middle chamber, and thus the entire translator (upper and middle chambers), to the lower chamber.

Spacers (Fig. 2b): These offset the lid from the upper chamber so that the lead screw is captured with tight tolerance (0.0005 in.). They have a 0.025 in. inner diameter, 0.040 in. outer diameter and are 0.0150 in. thick.

Upper chamber (Fig. 2a and Fig. 5b): This part contains six slots for the shuttles and a retaining lip for the lead screws, plus various screw holes. The slots are formed by electric discharge machining, all other surfaces are cut on a mill.

Shuttles (Fig. 2b and Fig. 5f): These translate along the length of the upper chamber and have two 0.010 in. holes for the electrodes, one in line with the inner circle and one in line with the outer circle of the guide-tubes in the array described below. The tight tolerances of the guide slots, and the large aspect ratio of the shuttles (approximately 1:2) result in very little tilt of the shuttles under load. This ensures linear and repeatable positioning of the electrodes.

Lead screws (Fig. 2b and Fig. 5f): These are 0.250 in. long and constructed from 0000-160 threaded rod that is glued (Superbonder 49950) to an hexagonal screw head dimensioned to fit a size 0 hex opening (0-80 set screw held by a fixture).

Middle chamber (Fig. 2b and Fig. 5b): This contains bore holes to capture the lead screws and set screws to clamp the array of guide-tubes in place.

Guide-tube array (Fig. 2b): A hexagonal array of 19 guide-tubes is made from 26 gauge tubes (Q-HXT-26; Small Parts) that are sheathed by an 11-1/2 gauge tube (Q-HXT-11-1/2; Small Parts). The array is constructed in steps, using a sequence of three jigs that are machined to capture seven tubes, 13 tubes, and all 19 tubes (Fig. 6a and b). At each step the tubes are carefully glued with low viscosity cement (Superbonder 49350), and the final assembly of 19 tubes is inserted into the 11-1/2 gauge tube and again glued. The assembled guide-tube array is cut and milled to an appropriate length. For the case of the vibrissa cortex, one end is milled with a curvature (0.180 in. radius) that matches the lateral–medial curvature of the dura at the position of the barrel fields (Chapin and Lin, 1984).

Lower chamber (Fig. 2a and Fig. 5d): This perimeter of this chamber attaches to the skull and provides a set for the guide-tube array. It has a small hole drilled into the side from which a short length (2 mm) of 20 gauge tubing (Q-HTX-20; Small Parts) is extended. During the implantation, vacuum is applied to the chamber via this port. For the case of the vibrissa sensory cortex, the bottom of this chamber is machined to match the curvature of the skull above the vibrissa, as with the case of the guide tubes.

Appendix B. Stereotrode fabrication

A single length (approximately 6 in.) of 25 μm parylene-coated tungsten wire is cut, the insulation is removed at both ends, the wire is folded and suspended with a small weight (e.g. 1/4 in. flat washer) at the fold, and the loose uninsulated ends held by two miniature electrical clips. The clips are connected to an ohm-meter to allow the conductivity between the two ends to be measured. The wires are twisted with an approximately 0.1 in. pitch and the pair is heated at $T \cong 250°C$ for approximately 90 s with a heat gun (HL1800E; Steinel, Germany) held vertically just below the washer. The folded end of the fused pair is cut at an angle with tungsten carbide edge scissors (no. 160-203; George Tieman, Hauppauge, NY) and the resistance between the two wires is measured to check for an electrical short, as occurs when too high a temperature is reached.

The cut edge is beveled at approximately 45° with a coarse diamond disk (BV-10 with 104C disk; Sutter Instruments, San Rafael, CA). Any wisps of the parylene coating that remain at the tip are removed by gentle wiping with lens paper. The tip is electroplated with gold as follows: Each free end of the

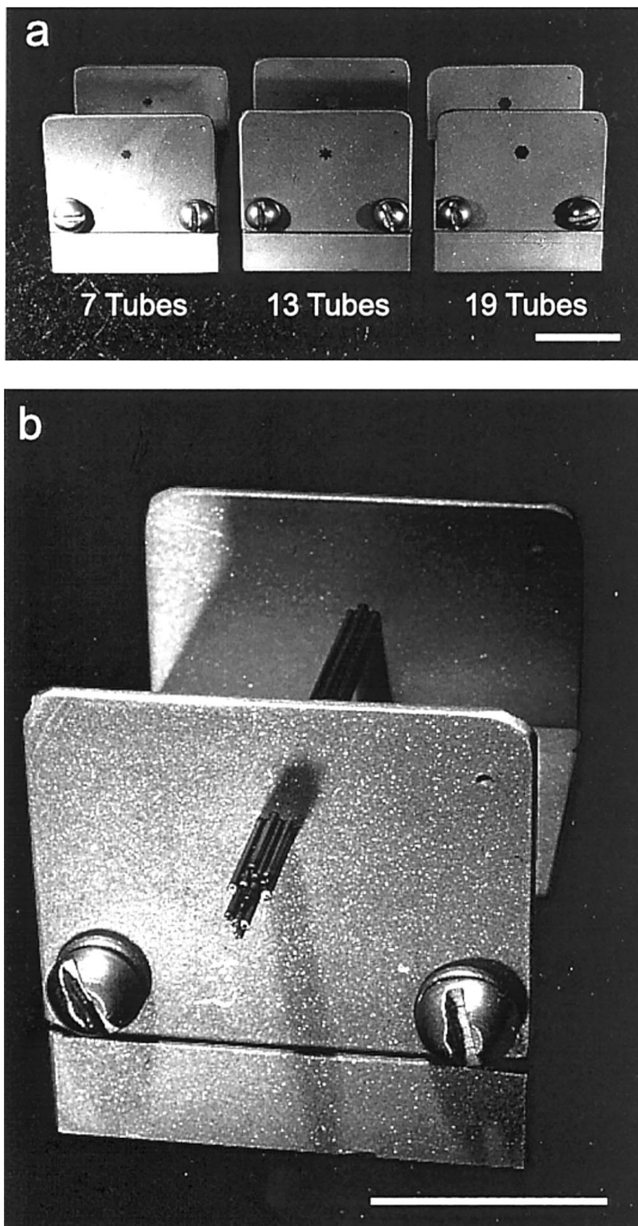

Fig. 6. Photographs of the jigs used to assemble the guide-tube array. (a) Three jigs, machined so that the openings correspond to the outer surface of a hexagonal assembly of seven tubes (left), 13 tubes (middle) and 19 tubes (right). The openings were cut with a 0.5 mm end mill at the positions for a hexagonal lattice with a 0.018 in. lattice spacing. (b) A partially assembled guide-tube array at the level of 13 tubes. The scale bar in both panels is 0.500 in.

cally cleaned in 1 M KOH (50°C) by passing constant cathodal current (−1 μA) for approximately 60 s, rinsed in distilled water, rinsed in 1 M HCl (50°C) for approximately 60 s, rinsed again in distilled water, plated in Acid Gold Strike RTU (0.25 troy oz/gallon; Technic, Anaheim, CA) by passing a cathodal current (−0.3 μA) for 200 s, and rinsed finally in distilled water.

Note in Proof:

The dimension shown in Fig. 5b and 5c for clearance of the filister screw heads should be 0.036 in., and not 0.035 in. as indicated.

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