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Chapter 2 Surgical Techniques for Chronic Implantation of Microwire Arrays in Rodents and Primates

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> And still we could never suppose that fortune were to be so friendly to us, such as to allow us to be perhaps the first in handling, as it were, the electricity concealed in nerves, in extracting it from nerves, and, in some way, in putting it under everyone's eyes.
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> **Luigi Galvani, 1791**

INTRODUCTION

The study of electrophysiology started with the work of Luigi Galvani (1737–1798), who was the first to provide evidence for the electrical nature of "the mysterious fluid" (at the time referred to as "animal spirits"). Galvani's nephew, Giovanni Aldini (1762–1834), continued this line of inquiry in 1803, using Galvani's and Alessandro Volta's (bimetallic electricity) principles together, despite the fact that Volta did not believe in animal electricity. Carlo Mateucci (1818–1868) in Bologna and Emil Du Bois-Reymond (1818–1896) in Berlin described the phenomenon called "negative variation" when a galvanometer showed an unexpected decrease in current intensity during muscle contraction. The study of the electrophysiology of the nervous system began when Julius Berstein (1839–1917) proposed his theory of the nerve impulse as a wave of negativity (membrane theory of the nerve tissue). Later, using a galvanometer with one electrode in the gray matter and one on the skull surface (or electrodes in different points of the external surface of the brain), Richard Caton (1842–1926) recorded a feeble current in the brain. In 1870, for the first time, Gustav Fritsch (1838–1927) and Eduard Hitzig (1838–1907) inserted an electrode in the dura of a dog brain and stimulated the motor area, generating movement in the contralateral side of the animal's body (Niedermeyer 1993; Piccolino 1998).

The work of these and many other scientists marked the beginning of the study of the electrophysiology of the nervous system, opening doors to the possibility of stimulating different areas of the brain through electrical current and subsequently recording the brain electrical activity. Improvements in electrode manufacturing, the advent of modern acquisition equipment, and better surgical and asepsis techniques have provided us the ability to chronically implant multiple electrodes simultaneously in several areas of the brain in the

same animal (Nicolelis, Baccala et al., 1995) and to study the interactions of populations of neurons (Nicolelis, Fanselow et al., 1997; Ghazanfar, Stambaugh et al., 2000). Upon the animal's recovery from surgery, we have been able to record simultaneously from different brain areas of mice (Costa, Cohen et al., 2004), rats (Faggin, Nguyen et al., 1997; Ghazanfar and Nicolelis 1997; Nicolelis, Ghazanfar et al., 1997) and nonhuman primate brains (Nicolelis, Stambaugh et al., 1999; Nicolelis, Dimitrov et al., 2003) for long periods of time, from a couple of months in rodents (Ghazanfar and Nicolelis 1997; Nicolelis, Ghazanfar et al., 1997) to up to years in non-human primates, such as owl monkeys (Nicolelis, Ghazanfar et al., 1998) and Rhesus monkeys (Nicolelis, Dimitrov et al., 2003).

These recordings are carried out under several different experimental conditions and behavior tasks (Kralik, Dimitrov et al., 2001; Nicolelis and Ribeiro 2002). With chronically implanted multiple electrodes, it is also possible to record different layers of the same area of the brain (Chapin and Lin 1984) and study spatiotemporal response of many neurons (Nicolelis and Chapin 1994). Microcannulae can be attached to the electrode arrays and are used to inject drugs in the areas of the implant during chronic experimental recordings (Shuler, Krupa et al., 2002).

Chronically implanted electrodes offer unparalleled advantages for correlating neuronal activity and animal behavior. In our lab, these techniques were developed in rodents and later adapted to primates. Over the last several years, there have been significant strides in making rodent implantations more reliable, faster, and easier. We have identified and resolved many of the issues that now permit larger neuronal yields that last longer. As a consequence of continuous improvement in techniques, the length of time required for surgery has been reduced. At the same time, over the last 14 years, we have developed a surgical technique adapted to the unique features of primates. This has made primate implantations routine and reproducible. Here, we will describe detailed technical aspects of the current surgical implantation approach used in our laboratory at the Duke University Center for Neuroengineering (DUCN). Such a surgical protocol has evolved and benefited from almost two decades of accumulated experience on chronic multielectrode neural recordings (Nicolelis, Stambaugh et al., 1999; Nicolelis, Dimitrov et al., 2003, Kralik, Dimitrov et al., 2001; Nicolelis and Ribeiro 2002).

DIFFERENCES BETWEEN RODENTS AND PRIMATES PERTINENT TO SURGICAL TECHNIQUE

The success in obtaining recordings from chronically implanted electrodes and how long they last depends fundamentally on the quality of the surgical implantation technique. The ability to open small craniotomies, only large enough to fit the electrode array with minimal bleeding from the bone and from the meninges in very small animals such as mice and rats, requires practicing the techniques several times before attempting to gather good data using

the implant of electrode arrays. This is especially true in cortex areas, which are close to the dura and are very sensitive to lesions.

The surgical technique for implantation that we follow in our lab is similar for all species, but each species has its own peculiarities. For instance, the mouse head is very small and the skull is thin, requiring a delicate technique. Mouse surgeries require very small screws, drill bits, and custom-designed electrode arrays. Because of the small head size and bone thickness, inserting more than two small arrays of electrodes and more than two fixation screws per animal is not recommended. Compared to mice, the rat's head is bigger and the bone is stronger, increasing the possibility of using bigger electrode arrays and reaching more cortical and subcortical areas. Because rats are stronger than mice, they can tolerate larger amounts of the acrylate used to secure the electrodes in place, which increases the number of arrays that can be inserted in the brain (Faggin, Nguyen et al., 1997; Nicolelis and Ribeiro 2002). Cortical and subcortical localization in rodents is based on commercially available stereotactic atlases.

Theoretically, implanting electrodes into rodents and primates should be very similar; however, in practice they are vastly different. For investigators accustomed to the hardiness of rodents and trained in rodent implantation techniques, performing similar procedures on primates can seem overwhelming. For instance, the commitment of time, personnel, and lab resources is much greater for primate surgery.

Furthermore, from the point of anesthesia, primates require much more attention during surgery. Whereas rodents require only monitoring of a few physiological parameters and infrequent injections, maintaining a primate under anesthesia is much more labor intensive. Thus, for our primate surgeries, one member of the surgery team is dedicated to monitoring the animal throughout. This higher level of anesthesia technique is akin to pediatric anesthesia and requires specific equipment, planning, and personnel.

Obvious differences in anatomical details include a thicker skull, thicker brain coverings, and a better-developed subdural space, all of which also influence the definition of the optimal surgical strategy for chronic multi-electrode implantation in nonhuman primates. The thicker skull requires forethought in terms of the appropriate electrode length. The brain coverings including the dura and pia are better developed, more variable in their thickness and at least the dura requires wide opening with microsurgical instruments for electrode penetration. We have found that the pia and arachnoid layers of primates are more variable, often tougher, and more prone to dimpling than rodents, sometimes necessitating microsurgical opening, as well as paying careful attention to be sure penetration has occurred. The primate brain is more prone to problems with swelling and retraction due to fluctuations in CO_2 levels in the blood that are primarily affected by ventilation of the animals' lungs. Plans must be in place to deal with these issues intraoperatively.

Overall, we have also observed that intraoperative neural recordings are more difficult in primates than they are in rodents. Much more attention must be paid to noise detection and reduction, especially because many more electrical devices necessary for anesthesia are involved.

Primates are more prone to infection than rodents and require more attention to sterile technique throughout, with sterilization of all the instruments, the use of sterile gloves and gowns, and draping of the surgical field. For those unaccustomed to working in a sterile environment for long periods of time, this can present unforeseen challenges and cost significant amounts of time.

In summary, primate implantations require a team approach. It often takes several days to weeks to prepare and coordinate one's lab prior to performing primate surgery, underscoring the critical importance of preoperative planning.

SURGICAL TECHNIQUES FOR RODENTS

Preoperative Supplies and Room Preparation

Typically, our rodent surgeries are performed on a surgery table in a regular room in the laboratory or in a designated surgical room. The table is kept clean and uncluttered and has the stereotaxic apparatus already installed (for rats we use the cat and small primates stereotaxic apparatus with a rat adaptor, and for mice, we use the stereotaxic apparatus for small rodents, both from David Kopf Instruments, Tujunga, California). Between the two stereotaxic bars, a rectangular platform made of plexiglass is glued to a height adjustable stage where a warm pad is placed. This apparatus is used for the anesthetized animal for the duration of the surgery and immediate postoperative recovery. Other essential pieces of equipment include a binocular surgical microscope, a dental drill already installed in a source of compressed air, an amplifier connected to an audio monitor, an oscilloscope, and a micropositioner. The table, the stereotaxic apparatus and the plexiglass platform are cleaned before the surgery with 70% alcohol or Asseptiwipes (wipes moistened in a solution of N-alkyl(68%C_{12}, 32%C_{14}) dimethyl ethyl benzyl ammonium chloride 0.125%, *N*-alkyl(60%C_{14}, 30%C_{16}, 5%C_{12}, 5%C_{14}) dimethyl ethyl benzyl ammonium chloride 0.125%, isopropyl alcohol 14.850%, and other ingredients.

All our surgeries follow the National Institute of Health (NIH) and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines for rodents that are expected to recover from anesthesia. These guidelines include appropriate preoperative and postoperative care of the animals, asepsis (sterilization of the surgical tools, use of sterile gloves, mask, head covers, clean lab coat, and aseptic procedures), gentle tissue handling, minimal dissection of tissues, effective hemostasis, and correct use of suture

materials and patterns when stitches are necessary (National Research Council 1996; Baumans, Remie et al., 2001).

The surgical instruments (basically, four small hemostats, scalpel handle, Freer periosteal elevator, scissors, micro scissors, regular and micro tweezers, dental drill handpiece, special screw drivers, stainless steel wire cutters, and micro cup curette) and supplies (gauze, cotton-tip applicators, kimwipes, drill bits, small stainless steel screws, silicone cups for dental acrylic, and beakers) are sterilized by autoclave. All supplies that cannot be exposed to heat and humidity are sterilized by ethylene oxide gas (extrafine point markers, plastic rulers, electrode holders, electrodes, etc.).

The microwire arrays (see chapter 1) are made of very delicate materials and cannot be sterilized by autoclave. Instead, these must be sterilized by ethylene oxide gas. The electrodes should be carefully packed in such a way that they are not damaged during handling, transportation to and from the sterilization facility, or by personnel helping in the surgery. Because of the length of these surgeries, all the equipment used during surgery should be routinely tested the day before the animal is anesthetized. To avoid unnecessary delays during surgery, it is imperative that all materials, equipment, and drugs for the surgery are readily available and in good working condition. A copy of the brain atlas appropriate to the animal undergoing implantation surgery is necessary to help in the location of the areas to be implanted. It is advisable to complete these steps the day before the surgery; however, they can be completed the day of the surgery prior to anesthetizing and preparing the animal.

Preoperative Animal Preparation

Typically, all animals selected to undergo chronic implantation of multielectrode arrays have several days to acclimate in the new laboratory facility before surgery. Often, these animals are subjected to weeks of behavioral training before they are implanted with arrays of electrodes. Only animals in good health are subjected to the surgery.

In general, we work with Long–Evans adult rats, males (in general 300–350 g) or females (in general 250–300 g). However, depending on the studies we can use younger or older animals. Mice are chosen according to their genotype since our laboratory currently utilizes a variety of mutant animals and their wild-type litter-mates as control (Costa, Lin et al., 2006; Dzirasa, Ribeiro et al., 2006).

Because rodents have a high metabolism and are not at risk for vomiting and aspirating, food is not withheld until up to 2 h before the surgery.

Plans for anesthesia, surgery, and determining the coordinates of the areas to be implanted must be carefully studied ahead of time. This will help decrease the time of the surgery for electrode-array implantation. A record sheet should be started for the procedure prior to

beginning anesthesia. This not only meets the requirements of AAALAC, NIH, and Institutional Animal Care and Use Committee (IACUCs), but also provides a record of the procedure, the implanted areas and their coordinates, the depth of the electrode for each array, and the position of the ground wires related to the position of the connector of the array.

Anesthesia Techniques and Intraoperative Monitoring

Following guidelines from Duke University's IACUC and the advice of Duke's veterinary staff, we use two anesthesia regimens for rat surgeries and one for mice surgeries.

For rats:

Pentobarbital IP

Ketamine associated with Xylazine IM

For mice:

Ketamine IM associated with Xylazine IM

Pentobarbital has a good hypnotic effect, but analgesia is obtained only when using high doses of the drug, which can cause cardiovascular and respiratory depression. The advantage of using Pentobarbital is that the effect of the anesthesia lasts longer than the Ketamine/Xylazine combination.

Ketamine is a dissociative anesthetic that provides light surgical anesthesia with a short duration. When combined with sedatives or tranquilizers, the quality of the anesthesia is highly improved and the duration of the effect is increased. In general, in our surgeries supplemental doses of Pentobarbital are required every 2 h and Ketamine every 1½ h. The supplemental doses for both drugs are from to ½ of the original dose, but Xylazine is not supplemented unless the surgery lasts longer than 7–8 hours, which is very uncommon in rodent surgeries.

For both anesthesia regimens (rats and mice), anesthetic induction is carried out in a chamber with a mixture of 5% Isoflurane and O_2. In our experience, a good induction with Isoflurane helps the selected anesthesia regimen last longer, decreasing the need for frequent injections of supplemental doses of the anesthetic. Once the animal is deeply anesthetized with Isoflurane, an injection of the selected drug is administered (Duke University DLAR 1995; National Research Council 1996; Hellebrekers and Booij 2001). Following the injection of anesthesia, the animals may receive a dose of Atropine SQ to prevent or treat excessive secretion of the airways and to improve cardiac function. This is important especially with the use of Pentobarbital.

For rats, if the surgery is expected to last for a longer period, small doses of sterile saline can be injected SQ, and IP injections of 10% dextrose solution will help to maintain hydration and blood glucose level during the surgery. In general, mice surgeries are much shorter than rat surgeries due to the small number of screws and arrays that are implanted, and extra volume or dextrose injections are not necessary.

Once the animal is deeply anesthetized, it is placed in an area for preparation for surgery, apart from the operating theater. The animal's head is shaved with small clippers, from the area just above the eyes to the back of the head and from ear to ear. If electromyography (EMG) electrode implants are planned, the skin on top of the target muscles is also shaved at this point. After the shaved hair is cleaned, the animal is transported to the surgery table and placed on the platform with a thermal pad to maintain the animal's body temperature throughout the surgery. If an electrical pad is used, the pad should be covered to avoid direct contact with the animal in order to prevent burns to the animal's skin in case the pad overheats, and a rectal temperature probe should be inserted. A good option is the Deltaphase® thermal pads (Braintree Scientific, Braintree, Massachusetts) that maintain temperature around 37°C for about 5–6 h. This type of pad must be monitored and changed when the temperature decreases.

After the animal is on a warm pad, it is mounted on the stereotaxic apparatus, using proper ear bars for rodents, placing the mouth and nose piece, and leaving the position of the head fixed until the top of the head is parallel to a flat surface.

Once the rat head is secure, the sterile pack can be opened and the sterile materials should be kept in a sterile field or container. The skin of the head is cleaned three times using Iodophor (iodine soaps) or Chlorhexidine followed by alcohol 70%. The animal's eyes are covered with an ophthalmic ointment to prevent corneal lesions since the animal loses the blinking reflex with the anesthesia.

Electrode Specific for Rodents

The arrays of electrodes to be implanted are made in-house and vary in number and distribution of electrodes. For the past nineteen years, our experiments have utilized arrays or bundles made of thin microwires (see chapter 1 for details). Some arrays can be made to reach more than one area of the brain.

In general, for rat surgeries, the arrays are comprised of 32 electrodes (4 × 8), but they can also be made up of either 16 (2 × 8) or 64 (8 × 8) electrodes. The tips are cut either blunt or sharp. When cut sharp, the electrodes may be able to penetrate the brain without dissecting the dura. In this case, the length of each electrode may be slightly different (see Figure 2.1). If necessary, a little cut on the dura with a bent fine needle may help relieve the tension of the

meninges and facilitate the implantation of the electrodes. Obviously, the length of the microwires varies for each surgery and depends on the brain area to be implanted.

Implantation Techniques

Brain Electrode Arrays

The length of the surgery for the implantation of microwire arrays varies from animal to animal. It also depends on the number of arrays to be implanted, the location of such brain regions, and the experience of the surgeon. In general, the minimum time is about 3–4 h if only one or two arrays are to be implanted. Each additional array can add about 45–60 min to the total surgical time. It is essential not to rush the implantation procedure. In our accumulated experience, gentle and slow penetration of the brain tissue yields the best long-term results.

During surgery, the status of anesthesia is checked periodically by pinching the inter-digit membrane of the hind paw or pinching the tails of the animal. Supplemental doses of anesthesia are given as needed.

The areas of the brain to be implanted also vary for each study and may vary even in the same study because it is not possible to reach all areas of interest for each animal, especially in mice. The surgical procedures described below have been very successful in the past. Following this approach has allowed our experimental animals to tolerate these implants very well, survive without any postsurgical complication, and provide high-quality recordings for months after the surgery.

After the animal is anesthetized, mounted to the stereotax, and cleaned, the surgical team suits up for surgery in a lab coat or gown, mask, sterile gloves, and head cap. Before starting surgery, the status of the anesthesia is checked as described above. To alleviate pain during the incision, an injection of lidocaine 1% or Bipuvocaine can be given under the skin at the site of incision. An incision is made on the midline of the scalp, from just above the eyes to the back of the head, and the skin is propped open. The borders of the skin are held open with small hemostats, and bleeding, which in general is very small, is cleaned with gauze or cotton-tip applicators. The periosteum is scraped off the skull bone using a blunt tool (Freer) or the scalpel blade. It is very important to clean the bone surface very well and remove all soft tissue attached to the bone. Bleeding spots on the bone may be cauterized or pressed until the bleeding stops.

The bone is then cleaned with hydrogen peroxide, applied very carefully to avoid touching soft tissues around the cut. This process can be repeated several times until the bone is clean, whitish, and all the blood is removed from the bone surface. The hydrogen peroxide is washed with sterile 0.9% saline, which is repeated as many times as necessary to remove the

excess chemical. The bone must be dried with gauze and must look very whitish. This step is most important for the fixation of the electrodes with dental acrylic. Any soft tissue or bleeding may lead to infection of the site, and the head cap may become dislodged some time after the surgery.

When the skull is clean and dry, an extrafine point marker, can be used to mark the skull. For rodents, the zero point is the center of the bregma. Using the stereotaxic apparatus, marks are made at the center of the area of the planned implants. The margins of the craniotomy for the electrodes are drawn around the central marks and other marks for the fixation screws are made. For mice, two screws are used; for rats, five to six screws are used. These metal screws (stainless steel, blunt tip) will hold the electrodes and the head cap in place and also provide a common electrical ground for the microwire arrays.

Using a dental drill under a surgical microscope view, small holes for the screws are then drilled. At least one of the screw holes must be completely open, exposing the dura. The screw placed in this hole will be in contact with the dura and will be used as a ground for the electrodes. The screws are placed in the holes and tightened with a screw driver, turning the screw enough to be firmly attached to the bone without penetrating the brain. If the dura is accidentally opened and cerebrospinal fluid (CSF) leaks from any hole after the screws are in place, it is recommended to either seal the screw with cyanoacrylate glue, or remove the screw and seal the hole with glue and place the screw in another location in the skull. Leaking of CSF under the head cap can lead to infection and softening of the bone over time, and will cause the head cap to become loose in the future, compromising the electrode positioning and increasing the risk of the head cap becoming detached from the skull.

After all screws are in place, the craniotomies for the electrodes are drilled. Care is taken to avoid damage to the dura during the drilling process. The size of the craniotomy must be just large enough to accommodate the electrode arrays. With a small drill bit, the edges of the craniotomy are drilled all the way to the bottom of the bone. To prevent damage to the dura, the bone should be tested frequently for softness. When the bottom feels soft, the rest of the bone can be taken out with a small cup curette, the tip of a needle, or with the tip of a dental explorer. Once all the bone from the edges of the craniotomy is drilled, the loose bone in the center can be removed either with a small sharp tool or micro tweezers. Once the craniotomy is opened, it is washed with saline until all the bone dust is removed. The skull needs to be dried again, and the craniotomy is covered with a piece of Gelfoam® moistened with saline or filled only with saline to prevent the dura from drying. The other craniotomies are opened the same way and covered with Gelfoam or saline. Drilling of the screw holes and all craniotomies should be done before the placement of the electrodes to avoid vibration of electrodes after they are implanted in the brain. If no damage is done to the dura, the craniotomy will look very clean and the surface of the brain and blood vessels can be seen.

Once all the craniotomies are opened, electrodes are set up for insertion, one array at a time. The Gelfoam is taken out of the craniotomy and is replaced by saline solution. The array, which is connected to headstages (cables and connectors) placed in special holders attached to the stereotax micromanipulators, is slowly lowered where the craniotomy is open to minimize brain damage and bleeding. Depending on how the tip of the electrodes are cut (sharp or blunt), the electrodes may be inserted in the brain without opening the dura. This maneuver is done slowly and with patience until the electrodes pierce the dura. If the wires are blunt, or it is impossible for them to break through, the dura must be opened. This can be done with a fine needle with a bent tip, taking care to avoid breaking the blood vessels. In general, a cut on the dura is enough to relieve tension, but it may be necessary to remove all the dura in the craniotomy.

After the electrodes are touching the brain surface, the special grounding wire is wrapped around the screws, including the screw reserved for grounding. The electrodes can be grounded in other ways. For example, the tip of the ground wire can be placed under the skull in a small hole, and this hole can be fixed with cyanoacrylate gel glue to secure the ground wire in place. Then the rest of the ground wire can be wrapped around the screws. However, this technique requires more time and patience. Cell activity should be monitored during the implantation of the electrodes to help ensure placement of the array in the desired layer or structure of the brain. If during the placement of the electrodes, the superficial blood vessels break and bleeding occurs, it is better to remove the electrodes from the craniotomy, press the hole until the bleeding stops, and wash the craniotomy and electrode tip with saline. In our rodent surgeries, bleeding in the dura is not cauterized. Blood on the tip of the electrodes may interfere with monitoring of the brain signals during surgery. A cotton-tip applicator over the craniotomy, a little roll of sterilized kimwipe or a piece of Gelfoam will help stop bleeding. Once bleeding stops, the craniotomy is extensively washed with saline, and the electrodes can be placed in the craniotomy again and slowly lowered into the brain. The electrodes can be lowered by hand with very small turns of the micromanipulators or with a micropositioner, about 100 μm at a time waiting a couple of minutes before proceeding with the lowering of the electrodes.

Dimpling of the brain may occur during electrode implantation, and it can result in traumatic brain injury. In our experience, this can be prevented by making the craniotomy as small as possible and performing the implantation slowly and gently, especially in cortical implants.

If the first insertion of the arrays is not successful and no signals are recorded, removing the electrodes and reinserting them in the same craniotomy does not work well for cortical areas, but may succeed for subcortical implants. In this case, the electrodes can be reinserted in the same craniotomy in a slightly different position.

After the array of electrodes penetrate the brain and reach the desired area (cortical or

subcortical), a layer of Gelfoam or warm agar is placed on the craniotomy around the microwires, being careful to avoid disturbing the position of the electrodes already in place. The excess ground wire is cut and the skull surface is cleaned and extensively dried again because cyanoacrylate glues or dental acrylic does not attach very well to wet bone. A drop of cyanoacrylate glue (gel) or dental acrylic is used to fix the electrodes to the nearest screws and to the bone, with care to avoid covering the next craniotomies. The craniotomy with the electrodes must be carefully sealed with acrylate to avoid leaking of CSF, especially when the dura has been surgically opened. After the glue or the dental acrylic is dried, the cables for recording (headstages) are removed from the electrode-array connector, and the electrode holder is gently released. If more electrode arrays are to be placed in the brain, the same procedures are repeated until all the arrays are in place. To decrease time during surgery, an accelerator can be used to speed curing of the glue when using cyanoacrylate glues.

After all the arrays are in place, a layer of dental acrylic is applied around and between all the arrays and screws to create a strong head cap fixed to the skull. The edges of the head cap should be smooth and should cover all of the bone to avoid postoperative infection. At this stage, a small piece of hard wire may be placed in the head cap (a staple will work very well for this purpose), which will be used to help anchor the head stage to the head cap with tiny rubber bands during future recording sessions.

If the skin is loose around the head cap, stitches may be necessary, and are done with suitable sutures. The skin around the cut area is washed with saline, dried, and a layer of antibiotic ointment is applied.

Once the surgery is over, the animal is returned to a clean cage and partially positioned under a heating lamp, such that when the animal awakes, it can move away from the heat if necessary. Care is taken to position the lamp to avoid burning the animal. The small particles of the bedding on the cage bottom can be covered with paper towels to prevent possible choking on or ingestion of the bedding when the animal is waking from anesthesia. The animal is checked at least every 15 min until it is fully awake. Moist food pellets and water are offered when the animal is fully awake and moving around. It is very important to observe the animal when it is waking, especially when Pentobarbital anesthesia is used. Secretion in the respiratory airways may cause the animal to choke during this period. If necessary, another dose of atropine may be given to prevent secretion formation and possible choking. All the animals subjected to surgery are housed in individual cages and followed closely for 7–10 days after surgery.

EMG Electrode Surgery

If the study requires recordings of muscle activity, EMG microwires (typically 50 μm tungsten, isonel coated) can be placed inside muscle pads of rodents. Muscle activity can be

recorded for many pairs of muscles. In general, we place 1–6 pairs of EMG electrodes in the following muscles: lavator labii superior, external epicantus, trapezius, biceps, triceps, and gastrocnemius. The EMG wires are attached to one connector (that will be attached to the head cap for the brain arrays) and are implanted during the same surgical procedure as the electrodes implantation in the brain. The EMG microwires are very thin and flexible, will not interfere with movements of the target muscle, and will not disturb the health and mobility of the animal. This step will add up to 30 to 90 min to the surgery depending on the number of pairs of muscles to be implanted.

After the brain electrode arrays are in place and fixed with dental acrylic, the shaved area on top of the target muscle is cleaned with iodine soap or Chlorhexidine and alcohol 70%. A small incision on the skin is made with a scalpel, and a long needle (20 Ga x 3½ in. disposable spinal needle) is inserted into the subcutaneous space on top of the muscle. The needle is pushed through the subcutaneous space up to the surgical opening on the head close to the electrode arrays. Once the tip of the needle is outside the cut area on the head, the EMG wire is passed through the needle until the tip is outside the body close to the target muscle. The needle is then removed, leaving the EMG wire in the subcutaneous space; the excess of the microwire is cut, and the tip inserted into the target muscle. The surgical opening is closed using suitable sutures. The area is cleaned and a layer of antibiotic ointment is applied.

These steps are repeated for all the muscles to be implanted with EMG wires. After all EMG wires are in place, the head cap is finished, and the cuts are cleaned and covered with antibiotic ointment.

Postoperative Care

In general, animals are fully recovered between 7–14 days following surgery, and they usually do not need any special procedure during postoperative days.

As a rule, our rodents are treated for pain for the first 24 h after surgery, whether or not they are showing signs of pain. Rats will receive a dose of Tylenol every 6 h or an injection of Buprenorphine every 8 h. Determining which to use depends on the status of the animal. If no signs or minimal signs of pain are present, the animals will be treated with Tylenol. If there is strong evidence of pain (animal is quiet in a corner, walking in circles, not eating or drinking, and has altered aspects of the fur and posture), the animal will be treated with Buprenorphine. Mice are treated for pain in the first 24 h following surgery with Buprenorphine. The animals are observed during the next postoperative days, and pain medication is given as necessary. Typically, our animals are back to their normal behavior and routine 24–48 h after surgery.

The surgical wound is checked everyday for signs of bleeding and draining, and to determine

whether bedding materials may be contaminating the cut. If wound care is required, the animals will be anesthetized in an Isoflurane chamber (5% Isoflurane and O_2 mixture), the wound will be cleaned and a new layer of antibiotic ointment will be applied. If the surgical area appears abnormally red or swollen, is draining fluid or if the animal does not appear to be recovering well, the animal will be treated with systemic antibiotics and pain medication will be given as necessary. After 10–14 days, stitches will be removed.

In successful surgeries, the animals tolerate the presence of the head cap extremely well and neuronal activity can be recorded for months.

SURGICAL TECHNIQUES FOR PRIMATES

Preoperative Supplies and Room Preparation

For nonhuman primates, the surgical area is divided into three rooms. One room is used for general preparation. This room contains head covers, masks, face shield protectors, and shoe covers for the lab staff involved in setting up the surgery and preparing the animal. This room also contains the table where the animal is prepared for surgery. The second room is used as the scrub room and contains a sink for the surgical team to scrub in before surgery, along with sterile garment supplies such as sterile gloves and gowns. The third room is the surgery suite.

Surgery at our institution is performed in a dedicated operating room (OR) equipped with OR lights, an operating table, a microscope, suction equipment, ventilation equipment, an oxygen and air supply, a ventilator, and a physiological monitor. The presence of suction equipment is important for venting of inhalational anesthetics. A variety of animal ventilators are commercially available. Not all ventilators work well with very small animals, such as owl and squirrel monkeys, due to the small volumes and pressures that these animals require. A physiological monitor that can display waveforms for heart rate, breathing, oxygen saturation, temperature, and EKG is the cornerstone to intraoperative monitoring of the animal.

Surgical instruments consist of basic clamps, scissors, and forceps. More important are the microsurgical instruments. A micro cup curette works well for trimming bone edges on the inside of the craniotomy after drilling. Micro scissors and micro forceps are needed for removal of the dura. Very fine suction catheters are useful to clear CSF from the operative field.

The surgical microscope should have binocular vision and must be covered with a sterile drape designed for microscopes. Magnification and ease of use are generally limited by cost. The microscope is used extensively for drilling craniotomies, dural resection, and visually monitoring the entry of the electrodes into the cortex.

A variety of medications should be prepared and dosed, based on the animal's weight, and loaded into syringes prior to the day of surgery, minimizing the need to calculate and measure at that time. These include:

1. Steroids—given perioperatively to reduce inflammation and brain swelling.
2. Antibiotics—given prophylactically before surgery and throughout the operation via IV.
3. Fentanyl—an opioid analgesic that is also used as an adjunct for maintenance of anesthesia with isofluroane. It works well to suppress respiratory drive in a hyperventilating animal.
4. Epinephrine and Atropine—for cardiac emergencies.

For details regarding veterinary anesthesia medications and supplies, please refer to Hellebrekers and Booij (Hellebrekers and Booij 2001).

For electrode sterilization, electrodes are carefully packed to avoid contact of the tips with the packaging and damage to the microwires. They are sterilized in an oxide ethylene gas chamber. This step must be completed before the day of surgery to allow adequate time for the sterilization cycle and process.

The success of a surgery depends upon a number of personnel working together in a coordinated fashion. It is useful to develop a "flight plan," so that everyone knows what to expect. A typical schedule of our primate surgery day is as follows:

- Animal arrival, last minute prep, attaching electrodes for EKG, O_2, and temperature monitoring
- Induction of anesthesia and intubation, placement of IV
- Fix the animal in the stereotaxic, create sterile field, drape microscope, drill, etc.
- Exposure and marking craniotomies
- Drilling of craniotomy and insertion of bone screws
- Lowering electrodes, allowing approximately 1 h per array
- Closure and waking of the monkey

Preoperative Animal Preparation

Only animals in good overall health should be considered for surgical implantations. Older animals with less cardiopulmonary reserve are more likely to develop postoperative problems such as pulmonary edema. We typically shave the animal's hair on the arms, chest, tail, and

head to allow easy application of electrocardiograph (EKG) leads and oxygen-saturation monitors. This can be done the day before surgery to save time. Food is withheld the day before surgery to lessen the chance of vomiting and aspirating into the lungs during administration of anesthesia and placement of the endotracheal tube.

Cortical localization should be planned well in advance. Atlas information and published coordinates of the areas of interest should be studied carefully. It is important to consider the depth of the cell layer of interest as well as the surface conformation—areas deep within a sulcus or very near the midline where the saggital sinus runs may be technically difficult to access. It is important preoperatively to have a three-dimensional idea of where the electrodes and connectors, head posts, and ground wires will fit on the surface of the skull. Electrodes with wires that are "offset" or angled may be necessary to access two areas, such as M1 and PMd that are close together. This issue is magnified when working with smaller species such as owl and squirrel monkeys.

Unlike rodents, there is more variability in the size, symmetry, and proportions of primate brains, making it important to have other backup methods in place to verify the locations of critical landmarks and structures. Preoperative magnetic resonance imaging (MRI) or computed tomography (CT) localization is one way to address this issue. In this paradigm, adapted from human functional-neurosurgical techniques, fixed markers such as a halo attached to the skull or implanted markers (e.g., under the scalp) can be referenced on scans relative to known sulci and gyri, thereby allowing the investigator to generate preoperative coordinates to find areas ofinterest (Scherberger et al., 2003).

Anesthesia Techniques and Intraoperative Monitoring

General anesthesia consisting of inhaled isoflurane through an endotracheal tube and supplemented by intravenous narcotics, generally fentanyl, is the mainstay of anesthesia. Details can be found in veterinary textbooks.

With early surgeries, we had difficulty with unpredictable swelling or retraction of the brain. Smaller species such as owl and squirrel monkeys seem more prone to this than rhesus monkeys. These smaller species have a tendency to hyperventilate when intubated and under anesthesia, resulting in blowing CO_2 and causing significant brain retraction. In order to address these issues, we have instituted use of a ventilator. End tidal CO_2 measurements are monitored continuously as they correspond well with swelling and retraction of the brain; CO_2 being the main driver of vascular dilation and constriction. We adjust the respiratory rate and tidal volume to keep CO_2 in the physiologic range. If brain swelling or retraction occurs, adjustments to the ventilation can be made to counteract this effect by manipulating the end tidal CO_2. Intravenous narcotics such as fentanyl are a useful adjunct to depress respiratory drive and counteract hyperventilation.

The various monitors and electrical devices used during surgery create a significant amount of electrical noise. Some devices can be run on batteries, thereby decreasing electrical noise. A strategy should be developed allowing basic monitoring that minimizes noise during the critical electrode-lowering phase of surgery.

Both core body temperature and depth of anesthesia are known to affect spontaneous neuronal firing. Core body temperature should be as close to normal as possible during the critical electrode-lowering steps when neuronal firing is being recorded. The depth of anesthesia is more difficult to tightly control. We have found that during the first steps of the operation, we can get a sense of a target heart rate that is indicative of an adequate level of anesthesia and a heart rate above which the animal is likely to begin moving. We titrate the anesthetic carefully during electrode lowering and neuronal recording to target this number. Other possible solutions to this problem include the use of paralytics to ensure the monkey does not move while temporarily lightening the anesthetics and simultaneously using ketamine anesthesia.

Electrode Specific for Primates

Although an in-depth discussion of various electrode designs can be found elsewhere in this book, it is worth mentioning some of the important general features of electrodes intended for primates.

In our paradigm, the electrodes are fastened to the skull rigidly after implantation, relying on the surrounding bone to maintain their place in the brain. The appropriate length of electrodes for smaller species such as owl and squirrel monkeys is 5–7 mm. For rhesus macaques, 6–8 mm is appropriate. One should expect variability in depth of the skull across different areas of the brain.

Implantation Techniques

Exposure

The surgical technique begins when the animal is stable under general anesthesia, fixed in the stereotaxic, and the skull vertex area is shaved, prepped, and draped in sterile fashion (see Figure 2.2). The skin incision can either be midline with extensions laterally to allow adequate exposure, or the skin can simply be cut in an oval pattern and removed—the scalp is very forgiving in its healing properties. Subperiostial dissection of the soft tissues is essential to assuring a good bond between the skull cap and skull. All soft tissues down to the cortical bone of the skull must be removed. Dissection laterally and posteriorly should extend well beyond proposed craniotomies. Laterally, the temporalis muscle and its attachments must be removed to expose hand somatosensory and motor areas. The soft tissues are then protected with gauze and tacked back using stitches to maintain the exposure. The convexity

of the skull is then prepped. Once dry, the border of the soft tissues and skull can be demarcated using a protective tape-type barrier to avoid leakage onto the soft tissues of the caustic materials used in the next step (see Figure 2.3). Next, hydrogen peroxide is used to remove all remaining shreds of soft tissue from the skull. Bleeding from the bone is stopped with cautery. Acid solutions and mechanical instruments such as a scalpel can be used to etch the outer cortical bone and to confirm that no soft tissue remains. The reason for doing this is to minimize or prevent a soft-tissue layer from developing between the skull cap and skull, which can be a nidus for infection and source of loosening of the head cap. The importance of careful skull cleaning cannot be overemphasized (see Figure 2.4).

Cortical Localization

The midline and interaural line is marked on the skull. Using predetermined coordinates based on these landmarks, the locations of the areas to be implanted are marked on the skull. Based on the size and orientation of the electrodes, craniotomies are outlined, taking into consideration how the connectors will be oriented. Bregma is highly variable in primates, and is not considered a reliable landmark for cortical localization (see Figure 2.5.)

Drilling of Craniotomies

Using a high-speed air micro drill adapted from dental applications, and the microscope, the craniotomies are drilled carefully respecting the dura. To verify the skull-based coordinates, appreciating that individual animals vary, it is a good idea to drill the first craniotomy near a prominent sulcus that is easy to distinguish. If the sulcus is not clearly identifiable through the dura, the dura is opened avoiding injury to any of the surface vasculature. Once the key landmark is found, the other craniotomies can be adjusted appropriately, if needed. This is a good backup mechanism to be sure that the areas of interest are definitively identified given the known variability in surface landmarks in primates (see Figure 2.6). An appropriately sized craniotomy should allow enough working space to be able to visualize cortical structures both for opening identifying landmarks and to facilitate the technical challenge of opening the dura as well as allowing visual confirmation, if possible, of electrode penetration. Thicker bone will warrant larger craniotomies to avoid working in a “hole.” A 1 mm margin around the footprint of the electrode is generally reasonable. Very large openings can cause significant leakage of CSF and distortion of the cortical surface, both of which are detrimental to the technique. Ideally, the opening allows space for the electrodes, while leaving the brain underneath in its native position. CSF shifts, or herniation of large craniotomies, may change the local mechanical forces enough to impede optimal electrode placement and stabilization. Too big a craniotomy also makes it difficult to secure electrodes, because the technique relies on rigid attachment to the surrounding cranium.

When all the craniotomies are drilled, securing skull screws are placed. A variety of options

exist ranging from small stainless steel screws available at the hardware store to specialized titanium skull screws for human applications. Although both work, the ease of use and time savings of the self-drilling, self-tapping titanium screws make them our first choice. “T-bolts” and other devices to secure the head cap to the skull can be employed to enhance security.

More important than discussing specific fixation techniques are the general principles that are important for obtaining secure fixation of the head cap. It is well known that if too much stress is applied to a bone or screw interface, the bone will gradually resorb, connective tissue will form, and the screw will loosen (Betelak, Margiotti et al., 2001). For bony integration to be achieved, for “oseteointegration” to occur, at least 3 months without significant mechanical stress is required (Worthington, 1994). Generally, 10–16 3.5 mm screws are adequate for holding a head cap in place but if a head post is required, more screws are necessary.

Because of spatial constraints, head posts used to fix animals during experimental sessions are often included in the same dental-acrylic mass as the electrode arrays. As described by Betelak et al., in such a paradigm, the screws must be specially positioned and time for osteointegration must be allowed (Betelak, Margiotti et al., 2001). If a head post is attached to the same head cap that holds the electrodes, a waiting period of 3 months should be taken into consideration in planning postoperative experiments with the head fixed.

Skull screws of any kind should be carefully tailored to the depth of the bone. Placing them too deep can have devastating consequences such as piercing the brain and causing bleeding, which can lead to seizures. Skull screws are used as grounds for the electrodes (see Figure 2.7). It is not advisable to drill craniotomies after opening the dura or after implantation of electrodes due to the bone dust that is distributed and the vibration that is caused. Spatial limitations make it nearly impossible.

Opening of the Brain Coverings

Unlike in rodents, we have found that it is always necessary to open the dura in primates to achieve successful penetration of the cortex. Under high-power magnification, the dura is opened in all of the craniotomies. A fine needle can be bent at the tip and used as a hook to slide under the dura and incise it. The dura has several layers and consists of white fibrous material arranged in overlapping stands. Care must be taken to be sure all layers have been opened. Freely flowing CSF and crystal-clear surface vasculature indicate complete dural removal. A cupped curette is useful to cut the dura off at its attachment to the edge of the craniotomy to allow full exposure. Rarely there is bleeding from the dura. This can be dealt with either with cautery or Gelfoam soaked in thrombin to enhance hemostasis. If there is bleeding from disruption of the cortical surface vessels, placement of a Gelfoam sponge

soaked in thrombin works well. Every effort should be made to avoid injury to the cortical surface. Opening the dura is challenging, and practice should be performed on rats to gain familiarity with the technique and to fine tune what works best in individual hands. At this point, the brain should be assessed and adjustments to the ventilation made, if needed, to correct any obvious retraction or marked swelling. A neutral brain that fills the opening of the craniotomy with occasional flow of CSF from under the craniotomy edge indicates a neutral position. If there is consistently even 1½ mm between the brain and the inner table of the bone, the brain is too retracted for optimal electrode insertion, and physiological adjustments should be made (see Figure 2.6). We have opened the pia through a variety of techniques including enzymatic digestion using collagenase, and mechanically with fine forceps. Although both are feasible and result in good recordings, despite visual disruption to the cortical surface, we have found that appropriate electrode spacing obviates the need to pursue these technically challenging and time-consuming steps. By adjusting the spacing of individual electrodes alone, we have been able to achieve consistent pial penetration. Other factors that can be manipulated to help achieve penetration are electrode diameter and the shape of the electrode tip.

Electrode Lowering

Once the dura is opened in all the craniotomies, the pia is protected from drying out with a small sponge (Gelfoam soaked in saline). The modified commercially available Kopf ™ stereotaxic arm is attached to the stereotax frame, and an electrode array is loaded, connected, and grounded to skull screws. The electrodes should be moving only in line with the direction of the lowering arm to avoid shearing forces upon entry. Every attempt is made to have the electrodes enter as perpendicular as possible to the surface of the brain realizing that the brain is not a flat surface. The order of electrode lowering is strategically planned. It is best to work outward from the middle craniotomy, thereby avoiding working in between delicate electrodes. Touchdown of the majority of the electrodes is marked as zero, and electrophysiological monitoring is begun. Noise issues are dealt with at this point. Anesthesia is titrated to target heart rate. The electrodes are then lowered approximately 100 μm at a time using microscopic visual guidance and electrophysiological monitoring of the electrode channels to determine entry into the brain (See Figure 2.8). A certain degree of dimpling occurs initially until the electrodes penetrate. We do not find any advantage to rapid or slow insertion. Occasionally, there is bleeding as cortical vessels are punctured if they cannot be avoided. This does not seem to affect intraoperative or postoperative recordings. Once characteristic firing of superficial cortical neurons is established in the majority of channels, the electrodes are lowered based on known depth of the target layer and most importantly electrophysiologic monitoring. We have found the quality of the intraoperative recordings, i.e., intraoperative single-unit firing, to be the most reliable predictor of good postoperative recordings.

Once the ideal location has been achieved, small pieces of cellulose sponges are placed around the electrode-brain interface. Cyanoacrylate glue in gel form is used to rigidly fix the electrode array to the skull (see Figure 2.9). The connectors are then removed, and the electrode is delicately released from the stereotax. This sequence is repeated until all electrode arrays are implanted.

Skull and Wound Closure

Once the arrays are all in place, being held by cyanoacrylate glue, there may be some leakage of CSF from the craniotomy openings. It is important at this point to seal the craniotomies in a water-tight fashion. The area to be covered by dental acrylic must be free of blood, soft tissue, or other debris that collected over the course of surgery. It must also be dry to allow the best possible bond to the bone. The better the interface between the dental acrylic and the skull, the less potential for CSF leak, development of soft-tissue migration, and infection. The acrylic is applied in layers until the electrodes are embedded up to the top of the connectors to protect them from breakage during the animal's normal movements (see Figure 2.10). Finally, a layer of antibiotic ointment is placed at the skull, scalp, and acrylic interface. Excess or devascularized skin is trimmed. Stitches or clips can be used to reapproximate skin edges, usually anteriorly and posteriorly. The scalp is very forgiving in its ability to heal.

Postoperative Care

Postoperative care first requires ascertaining cardiopulmonary stability, and personnel and equipment available to deal with such issues. If necessary, a warm water blanket is kept inside the animal's cage to maintain body temperature. Close observation for seizure activity and a plan for intervention are important. Seizures are infrequent and likely indicate an unanticipated bleed over the convexity, most likely from a misplaced skull screw. Neurological deflcits are surprisingly rare and generally resolve over time. The animals are usually returned to their cages by 24 h postop and advanced to a regular diet as quickly as possible. Post-op narcotics are used for the first postoperative day and in the subsequent postoperative days if necessary, however, narcotic drugs should not inhibit or delay the return to normal feeding, or depress respiration or other normal activities, which could potentially lead to further problems. After the second postoperative day, analgesia is carefully discussed with a veterinarian staff and if necessary is generally provided through NS anti-inflammatory drugs or Tylenol. However, narcotics will be used if necessary. It has been found with human surgery that, in general, early mobilization and return to function results in the least complications postoperatively, and the same principle applies to animal surgery.

The wound is cleaned when necessary, and a new layer of antibiotic ointment is applied. Stitches are removed from 10–14 days after surgery. Primates will be treated with antibiotics for 5 days after surgery.

CONCLUSIONS AND FUTURE DIRECTIONS

Analyzing and making use of neuronal data requires the critical first step of obtaining such information. The brain-electrode interface remains one of the rate limiting steps in our ability to advance neuronal-ensemble physiology. Techniques to acquire brain signals from rodents and primates have advanced rapidly, and this is only the beginning.

Over the last 18 years, we have worked on perfecting the surgical technique for implant of multielectrode arrays for chronic recording of brain cell activity. Our animals recover very well from the surgery, and they are usually back to their normal activities in 24–48 h without any signs of pain after the first postoperative day. Generally, there are no problems with postsurgical infection, and over time the animals are not disturbed by the presence of the head cap. However, we make every effort to continuously improve the surgical techniques for rodents and primates. Our goal is to be able to reach the relevant areas of the brain for each study with better and faster techniques, new materials, and identify new ways to improve healing of the tissues and skull bone, without compromising the efficiency of the recordings in the future. Especially for primate surgeries, preparation for surgeries involves extensive multipersonnel discussion of previous surgeries and ideas for future improvements. The design of the new electrode arrays, distribution of the microwires in the array, and materials used for the electrodes is carefully studied before each primate surgery and for each study involving rodents.

The use of mutant mice has provided a good data base for the study of several neurological diseases that affect many people in many different ways. Data collected and analyzed from chronic recordings in these animals have provided new insights in the mechanisms and physiopathology of conditions like Parkinson's diseases, depression, and psychoses (Costa, Lin et al., 2006; Dzirasa, Ribeiro et al., 2006). The possibility of studies on these animals is unlimited and may contribute to therapies or cures for progressive brain diseases that effect the lives of millions of people.

It is our goal to search for more biological-compatible materials for the manufacture of the electrodes and head caps, to develop wireless systems capable of recording brain activity, allowing us to study neuronal activity of animals in their regular environment, and to develop new methods for placement of electrodes in the brain that will result in shorter surgeries, less brain traumatic injury or lesions on the soft tissues and bone, and faster recovery from surgeries. Our ultimate goal is to find safer and better implantation techniques that can one day be applied to investigate and treat a variety of catastrophic neurological diseases that affect millions of people throughout the world.

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Figures

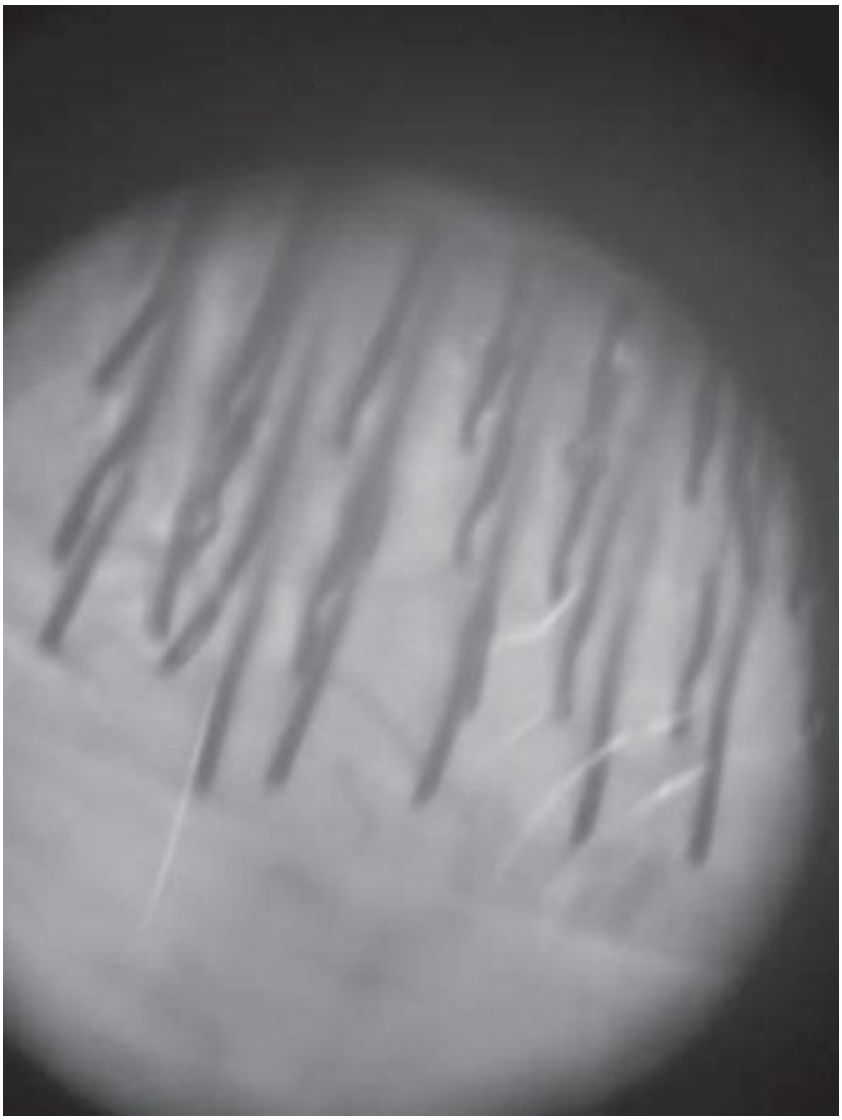

FIGURE 2.1

Sharp electrodes penetrating the dura in a rat surgery. Photo by Edgard Morya.

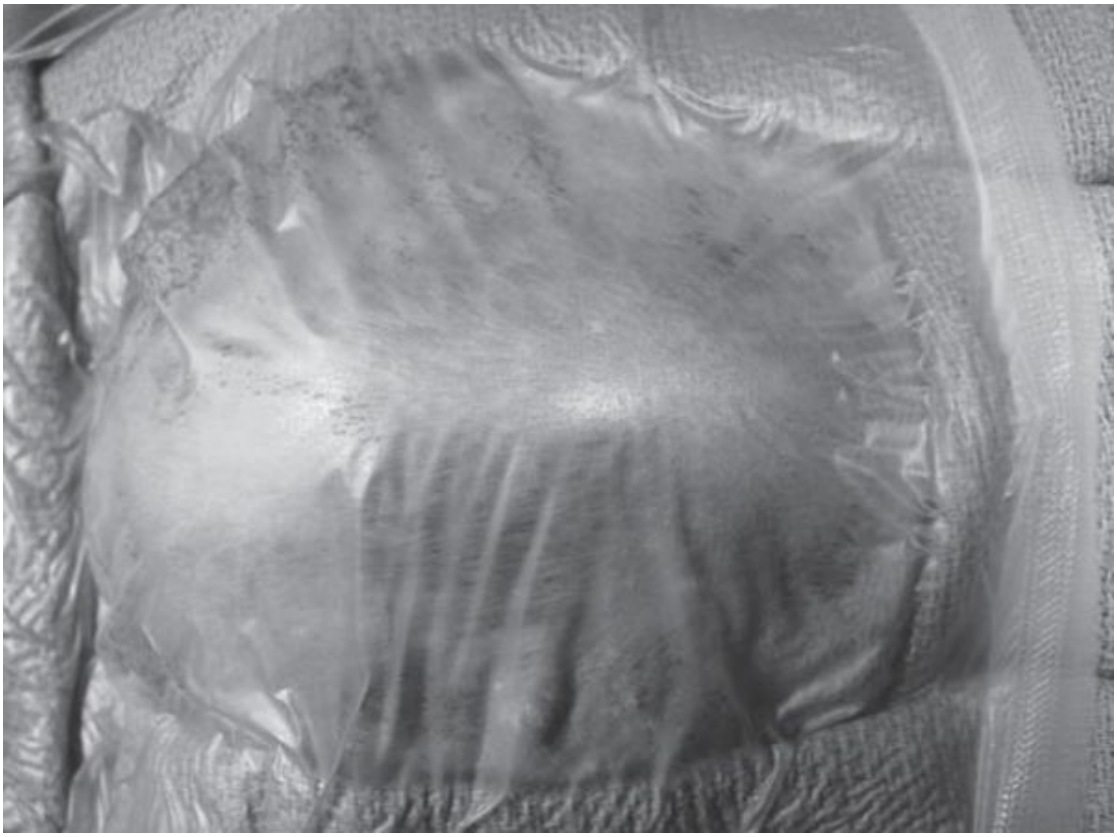

FIGURE 2.2

Rhesus monkey view of the vertex after sterile prep and drape is complete with Ioban ™ iodoform antibacterial drape on shaved scalp.

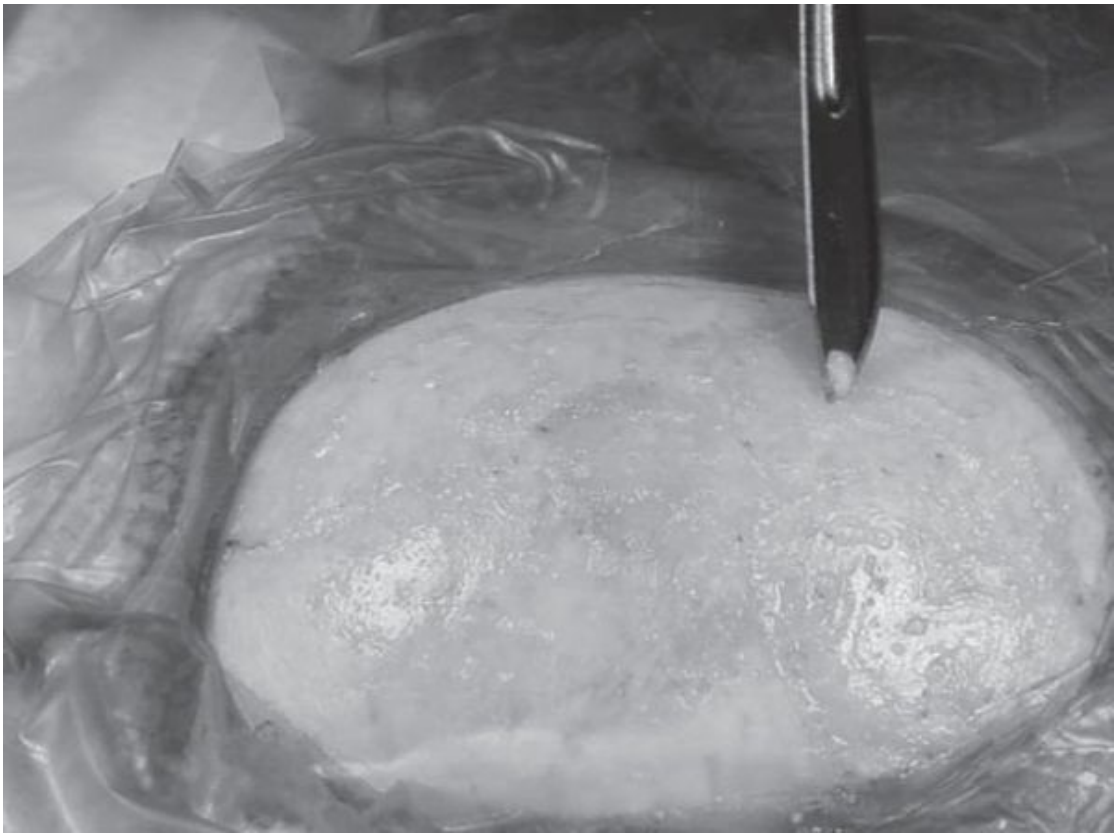

FIGURE 2.3

After removal of the scalp in an area wide enough to allow electrode placement, the skull is thoroughly cleaned with hydrogen peroxide and sharp instruments to remove all soft tissue from the scalp down to the outer cortex of the bone.

FIGURE 2.4

Skull surface after satisfactory cleaning. A bony lesion is evident in the center. All blood has been cleared. All periosteum has been removed.

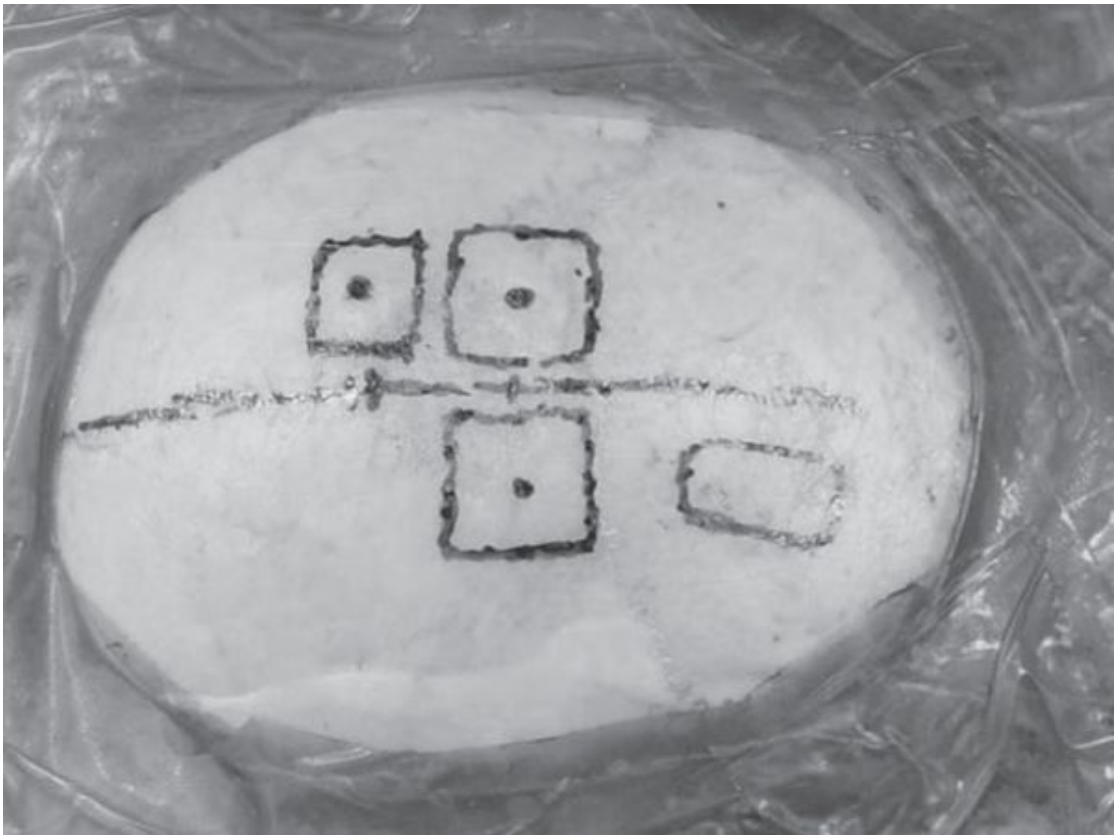

FIGURE 2.5

Predetermined coordinates are mapped onto the surface of the skull, in this case, based on interaural line and midline for the planned craniotomies, in this case bilateral M1 and left S1.

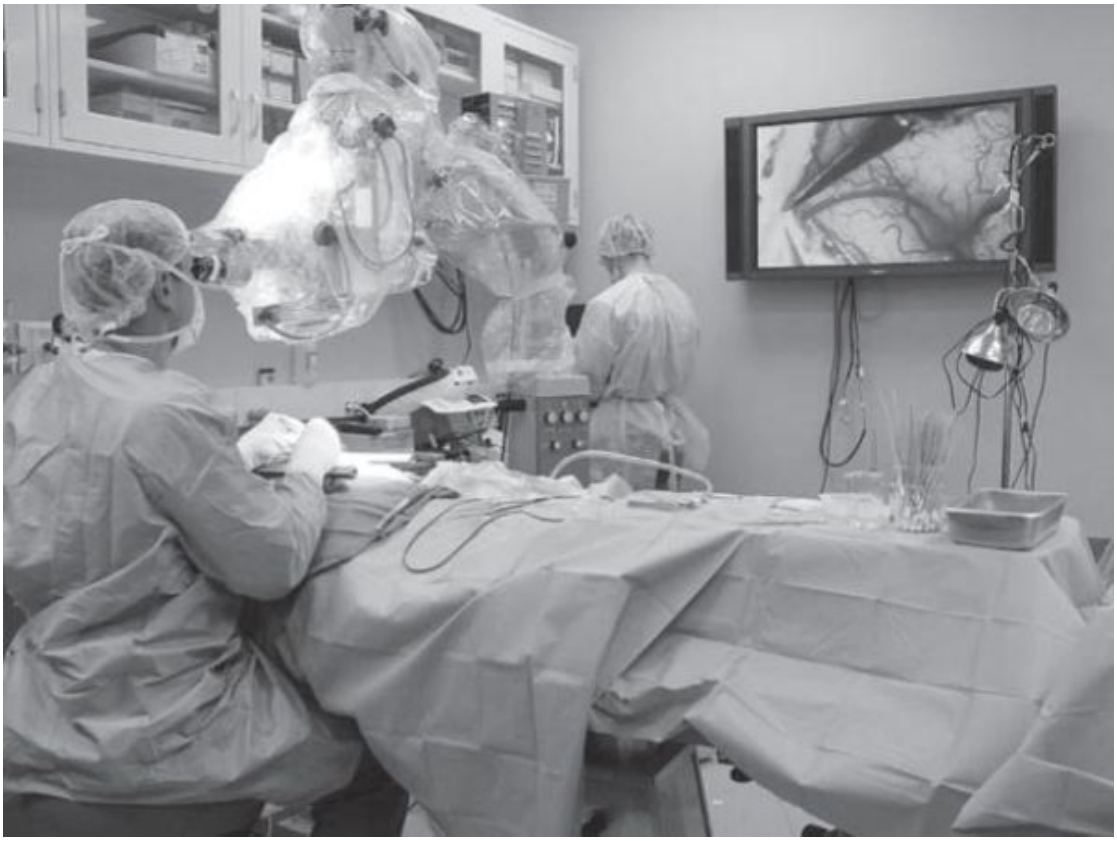

FIGURE 2.6

Operating room setup with sterile draping. Dura is opened microsurgically as depicted on projected plasma screen.

FIGURE 2.7

After drilling of the craniotomies and removal of the dura, M1 and S1 are evidently separated by the central sulcus on the left. On the right side, cellulose sponges fill the craniotomy defect. Skull screws have been placed for grounding and fixation purposes. Note that the final position of the craniotomies has been modified as the surface anatomy became clear during the course of the drilling. The craniotomies have been sized to accommodate the electrodes.

FIGURE 2.8

Electrode lowering proceeds under direct microscopic visualization with simultaneous electrode recordings to use both visual and neuronal firing to assess entry into the cortex and determine the ideal electrode placement.

FIGURE 2.9

Placement of the electrodes is complete. They are temporarily secured to the skull with cyanoacrylate glue visible in the center of the three electrodes. Ground wires have been connected to the skull screws.

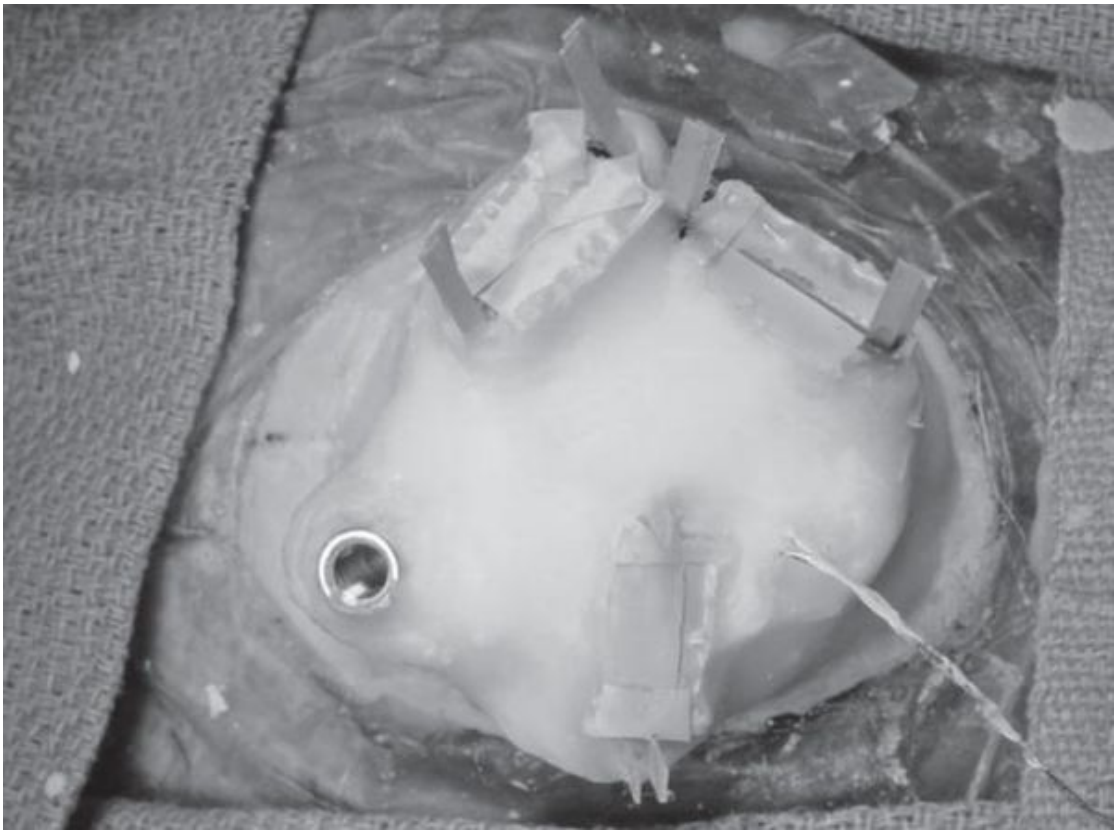

FIGURE 2.10

The electrodes are now secured to the skull and a head cap is created out of multiple layers of dental acrylic. The electrodes are completely embedded to protect them. A secondary grounding wire is visible. The interface between the skull and dental acrylic is smooth without intervening soft tissue at any point. No CSF leak is evident between the bone and head cap. Tape protects the electrodes during the cementing process. A thread is built into the head cap to allow a second protective cap to be fitted to protect the electrode connectors when not in use.

Bookshelf ID: NBK3895 PMID: 21204444