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Stereotaxic implantation of EEG electrodes in the rat

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Introduction

Electroencephalography (EEG) recordings in rodents are fundamental for researchers looking to study seizure disorders (i.e. epilepsy, status epilepticus), sleep and/or traumatic brain injury. While wireless telemetry has come a long way with regards to reliability and signal quality there is still the limitation of the number of channels that can be used for EEG (Pishan, 2011; Lin, 2010; Zayachkivsky, 2015). Wireless systems are capable of transmitting 2-3 biopotential sources (EEG, ECG, and EMG) via bluetooth or RF signal. A single channel of EEG would not be practical for those researchers interested studying and comparing bilateral and/or multiple EEG recording locations. A hard wired connection between the subject and the EEG machine can allow for numerous channels being recorded simultaneously. This article aims to provide a detailed methodology for the stereotaxic implantation of multiple screw electrodes used for recording multichannel EEG data from the surface of an adult rat brain. The electrodes rest on the cortical surface and are analogous to grid electrodes used in clinical intracranial monitoring. All procedures follow and comply with the National Institute of Health's (NIH) Guide for the Care and Use of Laboratory Animals: Eighth Edition (2011), Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and Institutional Animal Care and Use Committee (IACUC) policies, procedures and guidelines.

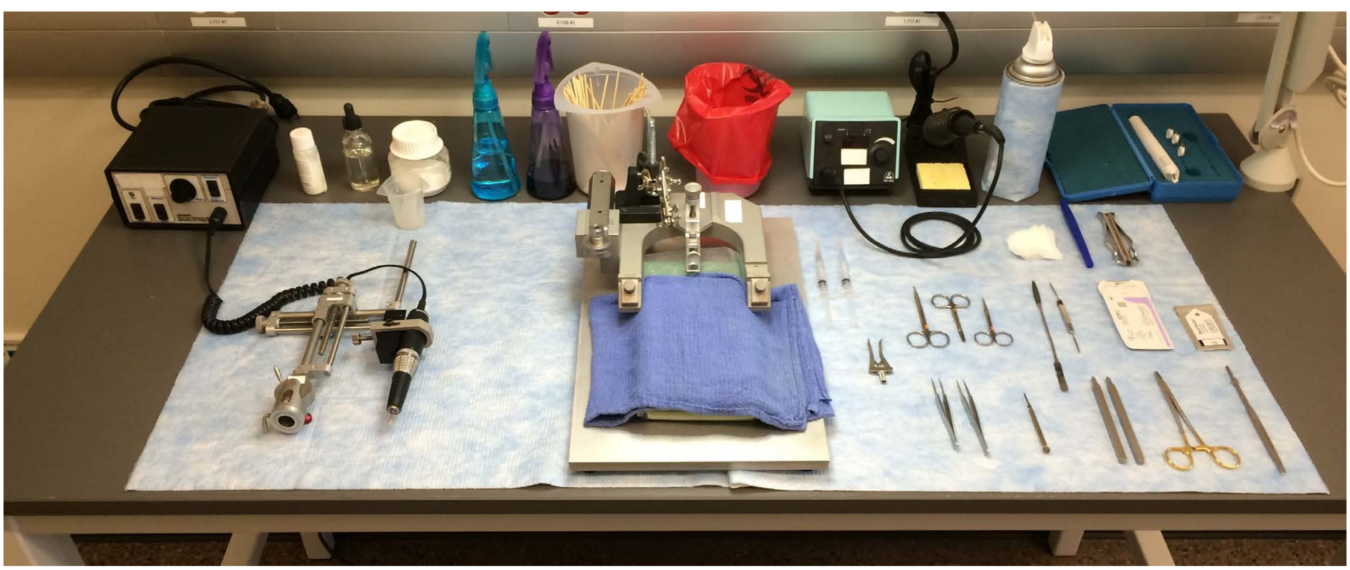

Figure 1. General surgical setup of materials and equipment required for implanting EEG electrodes.

Materials & Equipment

Gram Scale (Tanita, Cat. No. 1140)
Sprague Dawley rat (Envigo, Cat. No. 209M)
Rat restraint (Kent Scientific, Cat. No. RSTR544)
25 gauge syringes 1.0ml (Becton Dickinson, Cat. No. 309626)
Ketamine (Henry Schein, Cat. No 056344)
Xylazine (Vedco, Cat. No. 07-869-6707)
Acepromazine (Vedco, Cat. No. 07-869-7632)
Finishing Clippers (Oster, Cat. No. 078059-100)
Artificial Tears Eye ointment (Apexa, Cat. No. 704001)
Isopropanol (Fisher Scientific, Cat. No. S25372A)
Betadine (Purdue Frederick, Cat. No. 6505-00-286-7011)
45° Non-Rupture Ear bars (David Kopf Instruments, Cat. No. 955)
Stereotaxic frame (David Kopf Instruments, Cat. No. 900)
Stereotaxic arm (David Kopf Instruments, Cat. No. 960)
Versalon Sponge Dressing 2x2 gauze (Covidien, Cat. No. 8042)
Cotton tip applicators (Cardinal Health, Cat. No. C15055-006)
Scalpel Blade No. 15 (Bard-Parker, Cat. No. 371215)
Scalpel handle (Fine Science Tools, Cat. No. 10007-16)
Alm Retractors (Fine Science Tools, Cat. No. 17008-07)
Mosquito Hemostats (Fine Science Tools, Cat. No. 91308-12)
Stevens Tenotomy Scissors (Fine Science Tools, Cat. No. 14066-11)
Dumont Forceps (Fine Science Tools, Cat. No. 11231-20)
Laminectomy Forceps (Fine Science Tools, Cat. No. 211223-20)
Fine scissors (Fine Science Tools, Cat. No. 14060-09)
Spencer Ligature Scissors (Fine Science Tools, Cat. No. 14076-09)
Machined screws (Small Parts, Inc., Cat. No. 0-80 x ⅛)
Custom strip connector with relia tac pins (Marsh Designs, Cat. No. 401)
Burr drill bit (Meisinger USA, LLC, Cat. No. 1RF 012)
Foredom Micro motor (Foredom, Cat. No. MH1045)
Flat screwdriver (3/64” micro screwdriver)
Soldering station (Weller, Cat. No. WESD51)
Solder tip (Weller, Cat. No. ETS)
Nature-Cryl dental acrylic powder (GC America, Cat. No. 349774)
Nature-Cryl Pour dental acrylic liquid (GC America, Cat. No. 349779)
3.0 braided suture (Ethicon, Cat. No. J497G)
AutoClip 9mm clips (Thomas Scientific Apparatus, Cat. No. 7631)
Neo-Predef powder (Pharmacia & Upjohn Co., Cat. No. 146817)
Cauterizer (Fine Science Tools, Cat. No. 18010-00)
Saline (Braun Medical Inc., (Cat. No. L8001)
Hydrogen peroxide (Aaron Industries Inc., Cat. No. WM-68758G)
Skin marker (Fine Science Tools, Cat. No. 18000-30)
Helping hands (Allied Electronics, Cat. No. JA-40)
Buprenex Injectable (Reckitt Benckiser Healthcare, Cat. No. 7571)
30 gauge wire (OK Industries, Cat. No. KSW30R-1000)

Surgical Procedure

1. Have all material and equipment set up prior to surgery (Fig. 1). Record the rats pre-operative weight. To achieve a proper plane of anesthesia, a cocktail consisting of ketamine (50 mg/kg), xylazine (5 mg/kg), and acepromazine (1mg/kg) is administered at a dose volume of 1 ml/kg by intramuscular (i.m.) injection using a 25 gauge 1.0ml syringe. I.M. injection will provide about 60 minutes of anesthesia and is preferable over intraperitoneal which will provide only about 30 minutes of anesthesia. For post operative analgesia administer buprenorphine SR at 1.0 - 1.2 mg/kg i.m. using a 25 gauge 1.0ml syringe.

2. Once anesthetized, use the finishing clippers to trim all the hair from the top of the head. Use an air source to clear away any loose hair from the surgical site and eyes. It would be wise to drip lukewarm saline over the eyes to flush out any loose hair and then apply an adequate amount of ophthalmic ointment to the surface of the eye to prevent drying.

3. Affix the head into the stereotaxic frame by first placing the upper incisors over the bite bar, making sure the tongue and lower incisors fall below the bite bar. It is important the tongue is not sandwiched between the bite bar and roof of the mouth. Next set the ear bars, and use them to align the head so that the bridge of the nose is fairly well centered with the apex of the nose bar on the frame.

4. Being mindful of the eyes, clean the surgical field with 70% isopropanol. After the alcohol has air dried, wipe down the same area with a betadine solution. The nose bar can now be lowered onto the bridge of the nose.

5. Use the tip of the scalpel blade to penetrate the skin between the eyes, and then rock back so that the handle is almost parallel with the body. This will make for a smooth single incision back to between the ears. Drawing the blade back and simultaneously pushing skin forward through the blade will allow for a larger incision, without having to cut beyond the base of the skull.

6. With the tip of the scalpel blade cut and then scrape away the periosteum from the dorsal aspect of the skull. Once removed, place the micro-retractor to hold the skin away from the skull surface. Any residual blood can be removed with sterile cotton tip applicators and/or gauze. Sterile saline can also be used. Any small sources of blood can be cauterized to prevent excessive bleeding into the surgical field (Fig 2a).

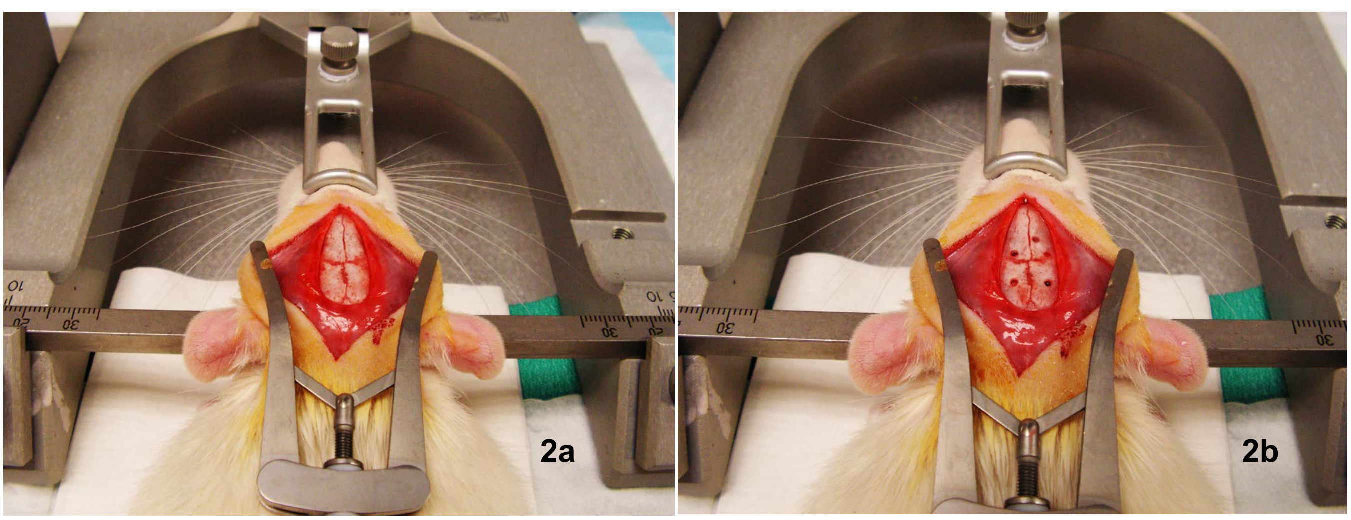

Figure 2. a. After removal of the periosteum the exposed rat skull shows both bregma and lambda reference points. b. Location of 5 craniotomies. Reference electrode (most anterior) site is chosen for its “quieter” signal relative to other cortical sites. Alternatively, some researchers chose a reference at the cerebellum for the same reason.

7. Once bleeding is controlled, mark bregma with the skin marker.

8. Next attach the stereotaxic arm with the Foredom drill to the frame and align the ball burr bit directly above bregma using the adjustment dials. The same style ball burr bit is used in oral surgery because it is suitable for cutting into dense material while leaving soft tissue unharmed. So the application for drilling bone, while minimizing damage to the dura is appropriate. Five 0.8mm diameter craniotomies are produced with a stereotaxic drill at the following coordinates from bregma: AP: 2.0mm, Lateral: ± 3.0,; AP: -4.0mm, Lateral: ± 3.0,; AP: 4.0mm, Lateral: + 1.0. Once properly positioned directly above the 1st electrode site, start the drill and lower it using the vertical dial on the stereotaxic arm. The thickness of an adult rats skull is about same as the diameter of the drill bit itself, just slightly less than 0.8mm. So as you're drilling and the bit is no longer visible, that means you have drilled deep enough and can back off the drill. Repeat this step with the remaining locations (Fig. 2b).

9. Epidural recording electrodes made from 30 gauge wire soldered to a #0-80 x 1/8 inch stainless steel screw will be implanted into each craniotomy (Fig. 3). These electrodes will serve two purposes. One is to conduct the electrical signal from the surface of the brain and the 2nd purpose is to anchor the entire headcap (connector and electrodes assembly) in place. Once the screw bites into the hole, apply 3 revolutions with the screwdriver. About half of the threads should still be exposed. There is no need to screw the electrodes flush with the skull. This would put unnecessary pressure on the brain. More importantly, at the end of this procedure we will be adding dental acrylic to hold everything in place; the acrylic needs to be able to get under the head of the screw to hold on more securely. If the electrode is screwed all the way flush, the dental acrylic will simply cover the head of the electrode like a cap.

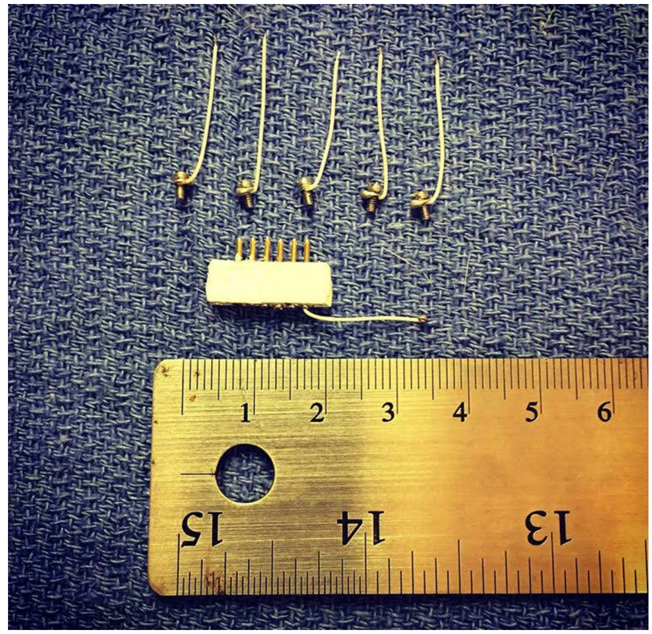

Figure 3. #0-80 x 1/8 inch stainless steel screws soldered to 1 inch strips of 30 gauge wire and a custom strip connector (male) with ground wire pre-soldered.

10. Using the alligator clips on the helping hands device, position the connector so that underside of all the pins are visible. Solder the implanted electrodes in order from front to back to the underside of the connector working from the right most pin to the left most pin. The reference electrode, being the furthest and most anterior, will be soldered to pin #1 on the right side of the connector. It is very important to note to touch the tip of the soldering iron to the solder in the pin and not the wire itself. Once the solder melts and looks like chrome push the wire into the pin. Touching the wire with the soldering iron will melt the insulation and worse, conduct heat down the the brain. The frontal left electrode will be soldered to the next pin to the left, followed by the front right electrode in pin 3. The rear left electrode will be in pin 4, while the rear right electrode will be in pin 5. A 1 inch long piece of ground wire should be soldered into pin 6 (Fig. 4). Opposite of the soldered end, use pair of forceps to grab the bare wire and twist it into a loop.

11. Once this is complete release the connector from the alligator clips on the helping

hands device, and turn it rightside up (pins facing upward) and in line with the body. Since the wires are insulated from each other, tuck all of them underneath the connector as tightly packed as possible.

12. Next place a few grams of powdered dental acrylic in a disposable beaker. Add a few milliliters of the liquid acrylic, just enough to get all the powder wet, but not soaking. This is a chemical reaction and the two components need to be mixed thoroughly. At first the mixture will be very runny so only apply a few drops to the sides of the connector allowing it to run underneath and over all the electrodes and wires. As the chemicals begin to cure in the beaker, it will thicken to the consistency of honey and then like toothpaste. It's during this phase that more is applied to build up the thickness on and around the headcap.

13. The acrylic will be fully cured after about 15 minutes. The nose bar can be then raised and the retractors removed. The ground wire can be tucked in the nape of the neck above the muscle and below the skin. Curl the wire so that is can freely bend as the animal turns its head (fig. 5).

14. The headcap will not allow for complete closure of the surgical site. To prevent the incision from tearing any further as well as keep the skin snug up against the headcap 3-0 braided suture will be placed at the anterior and posterior edges of the incision. A simple square knot with 3-4 throws on each will be sufficient (Fig 6). You'll want to be close to the incision edge, but not too close or you'll risk tearing the suture through the skin. The principle is to bring the skin together, but not smash it together with so much pressure that we restrict blood flow and healing.

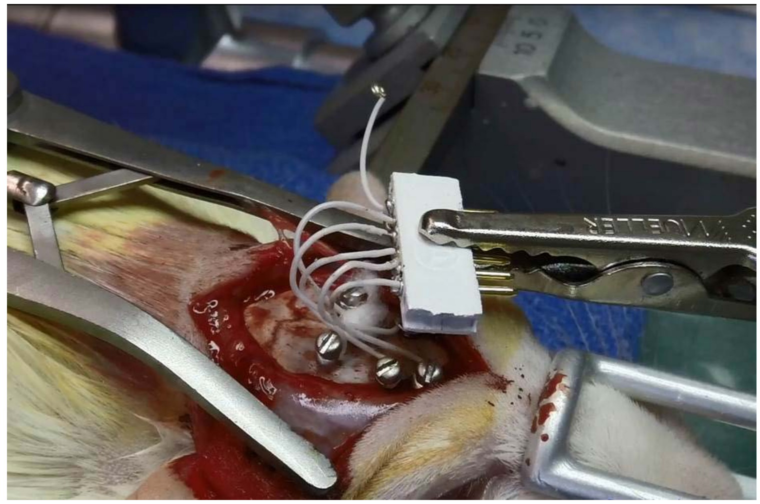

Figure 4. All electrodes soldered to the custom connector (male) and ground wire to the very left of the connector.

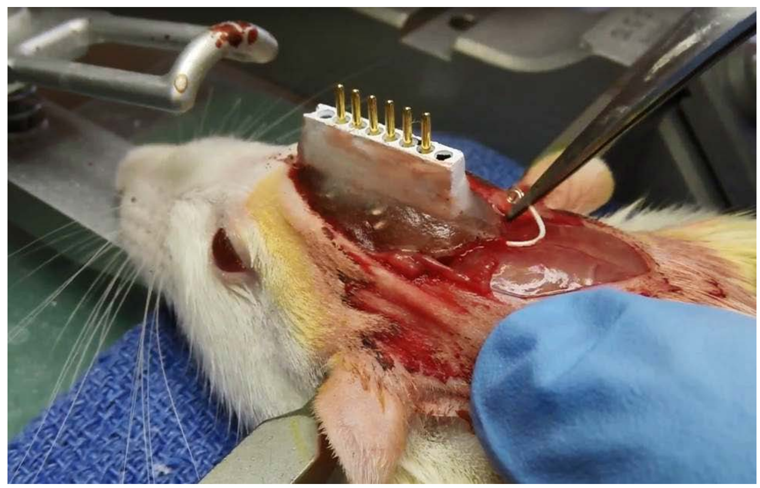

Figure 5. After the acrylic has fully cured, the ground wire can be curved and tucked under the skin. This curvature will allow the wire to move freely with the head of the animal and minimize discomfort.

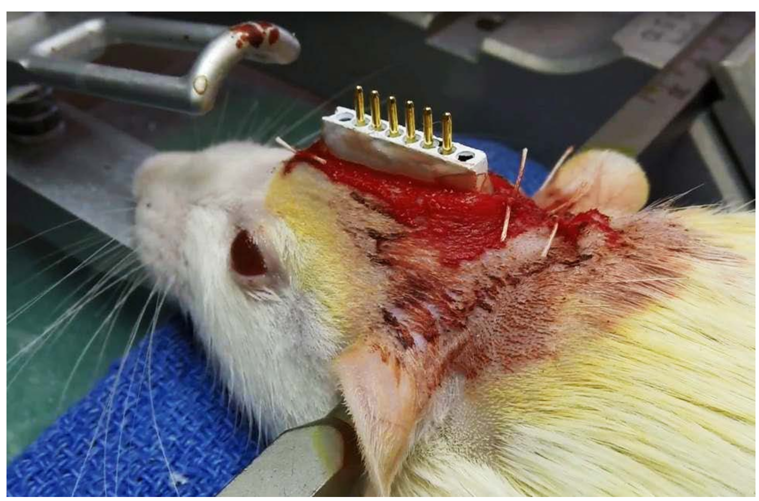

Figure 6. Completed surgical procedure. The surgical site should sutured snug to prevent debris from entering the surgical site.

15. Add an antibacterial powder that also contains anti-inflammatory and anesthetic ingredients such as Neo-Predef. This will aid in the healing process. To ensure the powder stays in place as long as possible take a 2x2 gauze pad, unfolding it once, then folding it over on itself lengthwise. Next wrap it firmly around the headcap and using the 9mm auto clip, staple the gauze pad to itself. This also aids with any residual bleeding as well as keeping bedding out of the surgical site. The ear bars can now be removed and the front top incisors up and off the bite bar. Let rat wake up in it's home cage, being mindful of the room temperature so it does not get cold. Monitor post operative care for next several days before connecting to an EEG machine for data collection.

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