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Chronic Multi-Electrode Assembly Implantation with a Chamber Setup

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Abstract

This protocol describes how to implant an array of carbon fiber and other types of microelectrodes for chronic recording using the two-part chamber system. The previous steps to implant the two-part chamber are described in other protocols entitled, "Baseplate Implantation for Two-Part Chamber System", and "Craniotomy, and Second and Third Phase Implantation to Complete Two-Part Chamber System".

Materials

Electrodes pre-assembled onto 3- or 6-channel microdrives (Gray Matter Research) and pre-threaded into 27G XTW guide tubes (Connecticut Hypodermics) filled with eye ointment (Puralube vet ointment, Dechra)
Custom-made stainless steel and Ag/AgCl reference wires
MRI-mapped grid (3D printed plastic) with 26G diameter holes at 1 mm pitch (Gray Matter Research). All the holes on this grid were prefilled with caulk (Kwik Seal Ultra, DAP 18916) and mounting screws
Cyanoacrylate (Rhino glue)
Acrylic cement (Jet Denture Repair Acrylic, Lang)
Acetone
Sterile paper point tips (HYGENIC #15-40 Assorted)
Sterile surgical tape (Medi-Pak Performance Plus DuoTip Marker with Ruler and 9 Labels, McKesson)
Screwdrivers
Custom-made electrode-interface board (EIB)
Tall protective cap (3D printed plastic) (Gray Matter Research)
Stereotax (Kopf Instruments)
Insulating varnish (GC Waldom 10-9002A Red GLPT Insulating Varnish)
Fast-scan cyclic voltammetry (FSCV) recording system (supplied by Scott Ng-Evans)

Note: All implanted materials and surgical supplies were sterilized on-site with ethylene oxide gas sterilization and/or hydrogen peroxide plasma from the vendor (Gray Matter Research).

Setup for Multi-Electrode Assembly Installation

1 Procedures were performed on Rhesus monkeys (n = 2) and were approved by the Institute's Animal Care and Use Committee (IACUC) at the University of Pittsburgh and were performed following the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services), the provisions of the Animals Welfare Act (USDA) and all applicable federal and state laws.

2 Monkeys were first given ketamine and atropine in their home-cage and then maintained on anesthesia with 1.5–2.0% isoflurane and 1 L/min oxygen. Analgesics, anti-inflammatory agents, and prophylactic antibiotics were administered pre- and/or post-op (i.e., meloxicam, dexamethasone, and ceftriaxone).

3 Monkeys were placed in stereotactic frames to fix their heads.

4 The protective cap was removed to expose the top of the chamber and its ports. The top of the chamber was disinfected by rubbing 70% isopropanol infused gauze across the surface several times. The surrounding chamber and implant margins were disinfected by wiping with gauze containing betadine and/or, separately, 70% isopropanol, in a sequential manner. Care was taken to avoid applying 70% isopropanol in the margins. A sterile field was created by isolating the top of the chamber with sterile drapes.

5 Electrode interface boards (EIBs) were installed onto the chamber for allowing subsequent electrical connection to implanted electrodes.

6 The port plug for the right hemisphere (i.e., the port in which craniotomy was previously performed) was removed from the chamber, along with the indwelling Kwik-sil plug. This exposed the brain surface (i.e., the surface of the dura mater and overlying granulation tissue). Only one hemisphere was targeted for this procedure, and the port targeting the opposite hemisphere remained closed, for use in future procedures.

7 The MRI-mapped grid was installed on top of the port with a rubber gasket and vacuum grease to reinforce the seal. On this grid, the holes in which electrodes would traverse to navigate towards targeted brain sites were marked with a black permanent marker to enhance visibility and facilitate manual alignment of electrode assemblies through these holes. All other holes on the grid were filled with caulk, except for the holes in which microdrive posts were to insert and attach into.

8 The side window cover of the chamber was also removed to allow for aspirating of liquid build up as a result of subsequent intracranial electrode insertion procedures, as well as to rinse 0.9% saline into the chamber when bleeding occurred. This also allowed us to visualize and confirm that the trajectories of inserted electrodes were perpendicular relative to the grid surface to reduce positioning errors.

9 Multiple (3 - 5) superficial Ag/AgCl and (3 - 5) stainless steel (SS) reference wires were inserted into the grid. The insulation on these wires had been stripped so that 2-3 mm of the conductor was exposed at the tip. These were positioned so that the exposed portions laid flat on the surface of the brain while remaining in the posterior edge of the chamber and outside of the electrode insertion fields.

10 3 - 5 intracranial Ag/AgCl wires were also installed to target the white matter area of the brain, and far away from brain structures targeted for neural recording. In this case, a 27G needle was used to gently penetrate the surface of the brain to create an opening in the dura mater. This was followed by inserting a 27G guide tube pre-threaded with the Ag/AgCl wire into the targeted area in the white matter. The Ag/AgCl wire was then pushed in deeper through the guide tube so that it's tip extended 3 - 5 mm from the guide tube tip so that the entire exposed portion of the wire was inside the brain.

Microdrive and Multi-Electrode Insertion

11 The targeted grid holes for the electrodes on a given microdrive were pre-penetrated to remove any caulk or debris residing in the grid hole, and to pierce the surface of the brain and create an opening in the dura-mater. This process was done before each microdrive installation.

12 The first surgeon positioned a microdrive with attached electrodes pre-threaded through guide tubes on top of the targeted holes above the grid.

12.1 The guide tubes remained on the electrodes without slipping as they were filled with eye ointment (Puralube), which helped keep these attached to each other. This lubricant gel was used to prevent fluid from inside the brain from channeling into the guide tube and outside of the chamber, and to maintain low friction between the electrode and guide tube to allow for further lowering of the electrode in future experiments through the microdrive screw mechanism.

12.2 The second surgeon helped support holding of the microdrive with tweezers. This was done to prevent the microdrive from falling or moving too much as the first surgeon lowered it manually onto the grid.

12.3 During the microdrive lowering, the guide tubes were first lowered and pierced into the brain. The electrodes remain inside the guide tubes and retracted from the tips to keep them protected during the dura mater penetration. Once all the guide tubes have advanced into the brain, the microdrive assembly continued to be lowered, and the electrodes and guide tubes together were slowly lowered into the brain.

12.4 Once the guide tubes reach their targeted depth, usually 5 - 10 mm above the electrode target, they are no longer lowered. The guide tubes were previously cut to discrete lengths based on their brain target so that when 2 mm of the guide tube remains above the grid, they are at their targeted depth. At this point, only the electrodes and microdrive were lowered together until the microdrive hits the grid surface and the electrodes reach their targeted depth.

12.5 Once the microdrive was securely attached to the grid (i.e., posts are fastened in the grid holes), the guide tubes were slowly lifted so that they remain just above the brain surface. Surgical tape was placed on the grid just in front of the guide tubes. This tape acted as a protective mask to prevent the liquid cement from covering unwanted holes. A paper point tip or applicator spear was used to apply a small amount of acetone on the guide tubes and the surrounding grid surface to remove any eye ointment residue, which can compromise the adhesive strength of subsequent acrylic cement. Then, cement was applied in the grid holes surrounding the guide tubes, between the guide tubes and the microdrive, and around the perimeter between the microdrive and grid to further reinforce attachment of the microdrive to the grid. The masking tape was then removed when the cement hardened, but before it was fully cured.

12.6 Electrodes were then connected to the EIB, which was connected to the FSCV amplifier headstage to allow recording of signals from the electrodes. Working electrodes were noted based on visualizing electrochemical current > 500 nA. Electrodes displaying current less than this threshold were noted as broken. The chamber was aspirated if needed and rinsed with 0.9% saline through the side window. This process (step 11) was repeated for each microdrive starting from the posterior edge of the chamber and adding microdrives one after another in the anterior direction.

13 Varnish was applied onto the mill-max connectors plugging in the electrodes on the EIB boards. This was done to maintain a physically robust connection and prevent future animal movements and vibrations from weakening the connection between the electrodes and the EIB boards, which is a common failure mechanism.

Installing Aspiration Port

14 An aspiration tube was installed onto the grid. This installation occurred during this procedure (monkey J) or 3 days after (monkey T). The aspiration tube consisted of a 23G (monkey J) or 26G (monkey T) needle attached to plastic tubing (10 - 15 cm long) using cyanoacrylate glue. The needle was inserted into the grid and several millimeters above the brain surface and then secured and sealed to the grid using Kwik-sil. Kwik-sil was used instead of a stronger adhesive (e.g., cement or cyanoacrylate) in the case that the needle was obstructed and had to be replaced in the future. The tube was closed with a stainless steel rod plug at the other end. Aspiration occurred after the electrode insertion procedure to remove liquid building up in the chamber.

15 The fluid inside the chamber was aspirated from the side window and then the window cover was screwed back on. A tall protective cap was placed to cover all the implanted electrodes.