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Individualized recording chambers for non-human primate neurophysiology

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Abstract

While neural recording chambers for non-human primates can be purchased commercially, these generic chambers do not contour to the animal's skull. In order to seal gaps, a cap of dental acrylic (methyl methacrylate) is often applied around the chamber. There are multiple disadvantages associated with this method. Applying acrylic delays and further complicates surgical procedure, and overheating during the curing process can cause damage to the bone. Post-surgery, acrylic margins can give rise to bacterial growth and infection. Here we describe a method to develop custom implants which conform to the individual's skull, thereby eliminating the need for acrylic. This method shortens surgery time and significantly improves the hygiene of chamber margins.

Keywords

primate; awake; behaving; chamber; headcap; acrylic; methyl methacrylate; PEEK

1. INTRODUCTION

Neurophysiology experiments in non-human primates often make use of recording chambers implanted on the skull. Generic chambers can be purchased commercially and are fastened to the skull using bone screws. However, as standard chambers are not contoured to the individual's skull, the interface between bone and implant is imperfect. The resulting gaps are typically sealed with a cap of dental acrylic (methyl methacrylate) (Betelak et al., 2001; Miyata et al., 1999). This method is not ideal for several reasons. Applying acrylic lengthens and further complicates surgical procedure: more screws are placed in the skull, the mold for the acrylic must be made, and it requires additional time to cure. Heat generation during the curing period is also an issue, and if excessive can cause bone damage or deter osseointegration with titanium implants (Ormianer et al., 2000).

Post-surgery, maintaining the cleanliness of acrylic margins is often problematic. The circumference of the acrylic head cap is larger than the implant, creating a larger area for the

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growth of granulation tissue. Rough or uneven edges may trap bacteria, which can lead to infection (Adams et al., 2007; Adams et al., 2011). Therefore, these head caps frequently require antiseptic cleanings every two to three days to prevent such an infection. Even if the margins of the head cap are well-maintained, changes underneath the acrylic can lead to its destructive failure. Often a spongy fibrous layer of tissue accumulates between the bone and the acrylic, eventually leading to separation of the acrylic from the bone (Betelak et al., 2001). In severe cases the bone under the acrylic simply dies, but whether this is due to mechanical changes or toxicity of the methyl methacrylate is unclear (Linder 1976). In any case, the lifetime of the implant and thus the window for optimal recording may be reduced.

Considering these various complications, it benefits both researcher and subject to eliminate the use of acrylic altogether. Tek et al. (2008) have presented a method of designing devices that can be rapidly prototyped, allowing for a quick and easy way to develop implants tailored to meet the needs of an experiment. The present study builds on the idea of custom design, and offers a method to create individualized recording chambers that conform to the subject's skull. First, the subject's MRI and CT data are co-registered, enabling identification of the region of skull above the target recording area in the brain. A reconstruction of the skull is then imported into a 3-dimensional CAD program where the implant is designed over the desired location. This creates an implant that conforms closely to idiosyncrasies of the skull, thereby eliminating the need for acrylic. This method shortens surgery time, and significantly improves the hygiene of chamber margins.

2. METHODS

All protocols were approved and monitored by the Arizona State University Institutional Animal Care and Use Committee and conformed to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996).

Four male rhesus macaque monkeys (*Macaca mulatta*) were used for this study. Monkeys I and J received implants based on a skull mapping chamber design (see below) while implants for Monkeys K and O were based on the MRI/CT chamber design. At the time of implantation, Monkey I was six years old and weighed 6.4 kg, Monkey J was 6 years old and weighed 5.6 kg, Monkey K was six years old and weighed 6.2 kg, Monkey O was 4 years old and weighed 4.9 kg.

2.1 Skull mapping procedure

In an early attempt to create a personalized chamber implant, "skull mapping" was used to define the contour of the skull by scanning the head with a stereotaxic needle in all three dimensions. After identifying the target location with a stereotaxic atlas, coordinates were recorded over a 4.5 x 3.5 cm area along 0.5 cm grid lines. The data points were then processed in MATLAB 6.1 (The MathWorks Inc., Natick, MA, 2000) for skull surface generation. This surface proved inadequate as the chamber followed a generalized form of the non-human primate's (NHP) skull, and did not match all of its irregularities. This led to the conclusion that MRI and CT data are necessary to design a truly personalized chamber that provides precise bone-to-implant contact.

2.2 MRI data acquisition

MRI data were acquired on a 3T GE Signa HDX magnet at the Keller Center for Imaging Innovation at Barrow Neurological Institute (BNI) at St. Joseph's Hospital in Phoenix, AZ.

The animal was induced with an injection of ketamine (10 mg/kg), intubated and catheterized through the saphenous vein and received a 1 ml bolus of propofol (10 mg/ml). A propofol drip at a rate of 1 drop/2 sec was set for maintaining the anesthetic plane. The

monkey was placed in prone position in a custom built stereotaxic frame which attached directly to the imaging coil. When available, radiopaque ear bars served as fiducial markers, although this was only provisional, as final data alignment was achieved using neural landmarks such as anterior and posterior commissures.

The full brain was imaged (16 cm field of view) using echo-planar imaging with a TR of 3D-T1 and voxel size of 0.6 X 0.625 X 0.625 mm.

2.3 Implantation of head-holding pedestals

Between the MRI and CT scans, head-holding pedestals were implanted. The animal was induced with 10 mg/kg of ketamine IM and 0.03 mg/kg of atropine IM. An IV catheter was placed in the saphenous vein; the animal was then intubated and placed on oxygen and 2% isoflurane. The surgical site was shaved and scrubbed with a chlorohexidine solution and alcohol. Preoperative care included 0.2 mg/kg of meloxicam SC, 0.02 mg/kg of buprenorphine IM, and then 50 mg/kg of cefazolin IV given over a period of five minutes. For the duration of the surgery, the monkey had a Lactated Ringers solution (LRS) drip at a rate of 5 ml/kg/hr IV.

Three titanium pedestals (Thomas Recording, Fig. 1A) were anchored to the skull using three 4 mm self-tapping Stryker bone screws. One “T” shaped pedestal was placed caudal of the supraorbital ridge and two “Y” shaped pedestals were placed rostral to the lateral margins of the occipital ridge. A small setscrew was inserted in the center of each pedestal to keep it clean. The incisions were sutured over the pedestals, which were allowed to heal for six weeks to encourage osseointegration (Fig. 1B).

Postoperative care included 0.08 mg/kg oxymorphone and 50 mg/kg cefazolin IM 8 hours after the perioperative dose and 0.15 mg/kg oxymorphone and 50 mg/kg cefazolin IM 8 hours later. Meloxicam (0.2 mg/kg) was given by mouth (PO) twice a day (BID) for three days and cephalexin (30 mg/kg) was given PO BID for seven days post surgery.

After six weeks, a small incision was made over the threaded center of each pedestal. The setscrews were removed and three posts (Thomas Recording), titanium rods required for our head-restraining system, were screwed into the pedestals (7 mm long in the “T” pedestal and 10 mm long in the “Y” pedestals). For this procedure the animal was sedated with a mixture of 10 mg/kg of dexmedetomidine hydrochloride and 5 mg/kg of ketamine IM and was given 2% isoflurane through a mask. When the procedure was completed, the monkey was reversed with 10 mg/kg of atipamezole IM. Perioperative care consisted of the same drugs and dosages as the pedestal procedure, except the cefazolin was given IM. Post-procedure, the animal received meloxicam (0.2 mg/kg) PO once a day (SID) for three days and cephalexin (30 mg/kg) PO BID for four days.

2.4 CT data acquisition

The CT data were acquired at the Veterinary Centers of America Animal Referral and Emergency Center of Arizona. The monkey was sedated with an injection of ketamine (5 mg/kg) and dexmedetomidine hydrochloride (10 mg/kg) IM, and reversed with 10 mg/kg of atipamezole IM. The animal was placed in prone position and a smear of barium cream was applied to the left side of the head for later orientation in the software; the titanium posts were used as fiducial markers for implant design. The resolution of the slice thickness was 2 mm in the coronal plane with a 1 mm overlap. The final voxel size was 0.3125 X 0.3125 X 2 mm. The animal's head was imaged at 16 cm, and these images were imported into Mimics v.13.1 (Materialize NV, <http://www.materialize.com/mimics>) to develop a 3D reconstruction of the skull.

2.5 Co-registration and target identification

The first step in co-registration required editing the data sets in Mimics to the same scale and slice thickness. The two data sets were then imported as DICOM images into Monkey Cicerone (Miocinovic et al., 2007) and aligned through translation and rotation of the neuroanatomical planes. Both MR and CT images were oriented in the workspace until the anterior and posterior commissures, as well as the ear and eye bars, were aligned with the same fiducial locations in the software's template (Fig. 2A).

Once the datasets were registered accurately, the target region for recordings was identified on the MR images. In the cases described here, the target was hand area of primary somatosensory cortex (SI). This target location was further confirmed by cross-referencing against both published and online macaque atlases (Paxinos et al, 2000; Saleem and Logothetis, 2007; <http://www.brainmuseum.org/specimens/primates/rhesusmonkey/>; <http://www.brainmap.wisc.edu/monkey.html>). The region of skull directly above this area was identified on the CT images for chamber design. The front post was used as a reference point, and measurements were made from the top center of the post to the target area of skull.

Distances were measured caudal and lateral relative to the frontal post. Graphic rulers designed in SolidWorks 2010 (Dassault Systemes SolidWorks Corporation) were imported into Cicerone to serve as an additional tool for measurement.

2.6 Implant design

A 3D reconstruction of the skull was produced from the CT data in Mimics and imported as an STL file into MeshLab (Visual Computing Lab ISTI- CNR), which was used to trim the skull to the portion required for the implant. The STL was then imported into SolidWorks as a "surface body" for chamber development. The desired location for the chamber was identified using the predetermined coordinates measured from the front post. It was built using the "extrude to surface" feature, which allowed customization of the chamber to specific skull shape (Fig. 2B).

The chamber was designed as a cylinder with four feet around its base, each foot varied in height due to the curvature of the skull. The cylinder has an inner diameter of 20 mm and an outer diameter of 27.94 mm. The top of the chamber remained level to support the electrode drive while the bottom matched the curvature of the skull, therefore its height varied from 24.89 mm to 12.7 mm (Fig. 2B).

The chamber was fabricated out of polyetheretherketone (PEEK) (PEEK-OPTIMA®, Invibio™), chosen over titanium because of its biocompatibility, machinability, and MRI compatibility. Furthermore, this material does not corrode or release metal ions, which can be the case with titanium (Sagomonyants, et al. 2008).

2.7 Verification in Cicerone

Once designed, the chamber was imported into Cicerone as an STL file. It was then positioned over the MRI and CT data to ensure the location was accurate, and that the bottom surface closely apposed the surface of the skull. A version of the chamber with electrodes aligned according to the electrode drive was also imported to aid in planning movable electrode tracks (Fig. 2C and D).

2.7 Implantation

The same anesthetic protocol as the pedestal surgery was used for the chamber implantation surgery. The animal's head was secured in a stereotaxic frame (Model 1430 David Kopf

Instruments), and its head was scrubbed with alternating chlorohexidine and alcohol. The stereotaxic coordinates found from the MR and CT scans were used to locate the desired area and an incision was made. Once the bone was exposed and cleaned, the stereotaxic coordinates were rechecked and the craniotomy was cut using a 15 mm trephine. Rongeurs were used to complete the craniotomy and extend the edges. The chamber was placed over the craniotomy and positioned to where it fit the skull perfectly; three 5 mm Stryker screws and one 6 mm Stryker screw were used to affix the chamber to the skull. The muscle and skin was sutured around the chamber. The monkey was removed from the stereotaxic frame and allowed to wake up and recover in its home cage.

Postoperative care varied slightly from the pedestal procedure. It included 0.08 mg/kg oxymorphone and 50 mg/kg cefazolin IM 8 hours after the first dose and 0.15 mg/kg oxymorphone and 50 mg/kg cefazolin IM 8 hours later. Meloxicam (0.2 mg/kg) was given PO SID for six days and cephalixin (30 mg/kg) was given PO BID for nine days post surgery.

3. RESULTS

This method has significantly shortened surgical procedure. Based upon records from 10 surgeries spanning the last three years, the process of applying acrylic including placing screws and set time, has taken an average of 30 minutes. Having a chamber that obviates the need for acrylic has reduced the amount of time the NHP is under anesthesia by 10%, decreasing the risk of complications.

As stated previously, acrylic margins pose a problem for bacteria and granulation tissue growth. Figure 1D shows an acrylic headcap 20 weeks post-implant. Note in particular the granulation tissue, rubor, and evidence of continuing aggravation around the margins of the implant, despite thorough daily cleaning. We observed scabs developing daily, the cleaning of which caused blood, and frequently pus, to discharge from the cap. In order to stop the bleeding, silver nitrate sticks were applied to cauterize the skin. This was obviously painful to the animal. Despite the time-intensive nature of maintaining this implant, the margins were eventually deemed too inflamed, and thus our animals with acrylic headcaps are routinely sedated about once a month for a vigorous cleaning. All scabs are removed, the perimeter is flushed with a chlorohexidine or betadine solution, the area is shaved, and silver nitrate is applied to the entire perimeter. This encourages cleaner acrylic margins for a longer period of time, and allows the researcher to get a deep cleaning otherwise inaccessible in an awake NHP.

By contrast, the margins of a customized PEEK implant 20 weeks post-implantation are shown in Fig. 1C. In this case, the perimeter is clean and devoid of aggravation. The overall implantation procedure required 3 hours and 20 minutes, from positioning the monkey in the stereotaxic frame to suturing the skin around the chamber. Note that the margins of this implant are clean, with no sign of inflammation. The chamber was placed with three 5 mm and one 6 mm Stryker self-tapping bone screws. Without acrylic, maintaining cleanliness of the implant takes considerably less time. In the 15 months since implantation, scabs have not formed around the perimeter of the implant and infection has yet to be seen. Currently, cleaning the area consists of wiping the margins with a chlorohexidine solution. Sedation once a month is unnecessary because buildup does not occur.

We also strived to design chamber feet that were lower-profile. The majority of these feet remain hidden under the skin, away from contaminated surroundings, yet the skin around two of these feet has receded. While the consequence of this is minimal, as the area remains generally clean, the researcher must now take care to fully disinfect the screw head and its surroundings.

In addition to guiding the creation of a personalized implant, acquiring MRI and CT data for the subject has aided the planning of neural recordings. Monkey K participated in a neurophysiology study involving single-unit recording in hand area of somatosensory cortex (SI) during performance of a reach-to-grasp task. To prepare for this, an STL file of the chamber with electrodes aligned according to the electrode drive was imported into Monkey Cicerone. After being aligned with the data in the correct stereotaxic location, a target was chosen in chamber coordinates that appeared optimal for driving an electrode into the hand representation area in somatosensory cortex. On the first day of recording, these coordinates produced a neuron with receptive fields on the thumb. Subsequent recordings were also successfully obtained in hand area of SI.

4. DISCUSSION

In this paper, we present a series of steps for creating customizable neural recording chambers. Our method uses MR and CT images to locate brain structures and create a 3D rendering of the skull prior to designing the implant.

Using this method, we have been able to generate recording chambers that conform to the specific contour of the individual monkey's skull. This has eliminated the need for using methyl methacrylate to seal the gaps between the chamber and the skull, which are usually present after implanting commercially produced recording chambers. This method has greatly improved hygienic maintenance, both in time and effort. Because of the lack of granulation tissue buildup, cleanings are less painful, which helps to keep the animal calmer for subsequent behavioral performance and recording.

The material used to construct the implant is also important for the success of these implants, specifically for the absence of granulation tissue around the margins. PEEK does not cause foreign body reactions around the implant site and is more biocompatible when compared to titanium (Nieminen, et al., 2008).

The availability of individualized MRI and CT data has furthermore improved accuracy in locating targets for neural recordings. While rhesus brain stereotaxic atlases are used for surgical and recording planning, consideration for individual differences is not taken into account. Monkey Cicerone, however, allows for the visualization of electrode tracts for the individualized chamber, and has thus allowed for a more credible discernment of recording sites. Its efficiency has been demonstrated during experiments, where the location of receptive fields has matched the anticipated outcome.

The longevity of the implant remains to be seen. In an early-phase trial of this method described in section 2.1, implantation required four bone screws and was acrylic-free, but after six months the implant came loose and had to be rotated and reattached in a second procedure. Since there was a small gap between the implant and bone, skin and hair began to grow inside the implant over the surface of the dura. We attribute these failures to the imperfect interface between implant and skull. We believe these issues have been resolved as the CT dataset provides a replication of the monkey's skull, therefore allowing precise bone-to-implant contact.

Implant failures may also result from the number of bone screws used to secure the chamber on the skull. For both the skull mapping and CT-mapped designs, we determined that four screws would suffice for an implant that was 20 mm in diameter. Although a greater number of screws might offer extra stability, we hoped to minimize the potential for aggravation. While we have eradicated the growth of excess granulation tissue and scabs around the implant, there is still some minor inflammation around the feet of the implant. Efforts to avoid such aggravation included using a minimal number of bone screws.

After 15 months the newly designed implant remains robust. Hygienic conditions have been optimized, and we are expecting the implant to endure the duration of the experiments, which will persist for another year.

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References

- Adams DL, Economides JR, Jocson CM, Horton JM. A Biocompatible Titanium Headpost for Stabilizing Behaving Monkeys. *Journal of Neurophysiology*. 2007; 98 (2):993–1001.10.1152/jn.00102.2007 [PubMed: 17522180]
- Adams DL, Economides JR, Jocson CM, Parker JM, Horton JC. A watertight acrylic-free titanium recording chamber for electrophysiology in behaving monkeys. *Journal of Neurophysiology*. 2011; 106 (3):1581–1590.10.1152/jn.00405.2011 [PubMed: 21676928]
- Betelak KF, Margiotti EA, Wohlford ME, Suzuki DA. The use of titanium implants and prosthodontic techniques in the preparation of non-human primates for long-term neuronal recording studies. *Journal of Neuroscience Methods*. 2001 Nov 15; 112(1):9–20.10.1016/S0165-0270(01)00442-3 [PubMed: 11640953]
- Linder L. Tissue Reaction to Methyl Methacrylate Monomer: A Comparative Study in the Rabbit's Ear on the Toxicity of Methyl Methacrylate Monomer of Varying Composition. *Acta Orthopaedica*. 1976; 47(1):3–10. <http://informahealthcare.com/doi/abs/10.3109/17453677608998965>.
- Miocinovic, S.; Noecker, AM.; Maks, CB.; Butson, CR.; McIntyre, CC. Monkey Cicerone: stereotactic neurophysiological recording and deep brain stimulation electrode placement software system. In: Sakas, Damianos E.; Simpson, Brian A., editors. *Operative Neuromodulation*. Vol. 97/2. Vienna: Springer Vienna; 2007. p. 561-567.<http://www.springerlink.com/content/p77224204r68835u/>
- Miyata GO, Fetissov S, Meguid M. A Complication of Long-Term Brain Microdialysis. *Nutrition*. 1999; 15 (9):723–724. [PubMed: 10467622]
- Nieminen T, Kallela I, Wuolijoki E, Kainulainen H, Hiidenheimo I, Rantala I. Amorphous and crystalline polyetheretherketone: Mechanical properties and tissue reactions during a 3_year follow_up. *Journal of Biomedical Materials Research Part A*. 2008 Feb 1; 84A(2):377–383.10.1002/jbm.a.31310 [PubMed: 17618477]
- Ormianer Z, Laufer BZ, Nissan J, Gross M. An investigation of heat transfer to the implant-bone interface related to exothermic heat generation during setting of autopolymerizing acrylic resins applied directly to an implant abutment. *The International Journal of Oral & Maxillofacial Implants*. 2000 Dec; 15(6):837–842.
- Paxinos, G.; Huang, XF.; Toga, AW. *The Rhesus Monkey Brain In Stereotaxic Coordinates*. Academic Press; Orlando, Florida: 2000.
- Sagomonyants KB, Jarman-Smith ML, Devine JN, Aronow MS, Gronowicz GA. The *in vitro* response of human osteoblasts to polyetheretherketone (PEEK) substrates compared to commercially pure titanium. *Biomaterials*. 2008 Apr; 29(11):1563–1572. [PubMed: 18199478]
- Saleem, KS.; Logothetis, NK. *A Combined MRI and Histology Atlas of the Rhesus Monkey Brain in Stereotaxic Coordinates*. Academic Press; London: 2007.
- Tek P, Chiganos TC, Mohammed JS, Eddington DT, Fall CP, Ifft P, Rousche PJ. Rapid prototyping for neuroscience and neural engineering. *Journal of Neuroscience Methods*. 2008 Jul 30; 172(2): 263–269.10.1016/j.jneumeth.2008.03.011 [PubMed: 18565590]

Figure 1. Implant devices

A. Titanium pedestal before implant. B. Titanium pedestal post-implant with osseointegration. C. A custom designed PEEK implant. D. A typical implant with acrylic support.

Figure 2. Chamber Design Sequence

A. MR dataset (red) and CT dataset (green) co-registered using the anterior and posterior commissures (green spheres under corpus collosum), and ear and eye bars (orange) as landmarks. B. CT reconstruction was imported as a mesh into SolidWorks, and the chamber was extruded to the contour of the skull. The purple ball represents the front post that was used as a reference point. C. In Monkey Cicerone, the chamber is shown in position without the CT data to show penetrations into the hand area of primary somatosensory cortex. D. Same chamber placement shown with CT data, verifying apposition of the chamber to the skull's surface.