Title: Custom-made implants for chronic in-vivo electrophysiological recording from primate's brain based on reconstructed skull model

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Highlights

- We introduced a customized design for affordable implants used in electrophysiology
- Enhancement of animal welfare with significant reduction of surgery time and chance of infection
- Our implants remain well-integrated with skull after two years.
- Optimized planning of skull area to access brain regions and guide craniotomy were provided by applying reconstructed 3d model of skull.

Abstract

Background. In-vivo neural recordings from primates require to install implants on the skull of the animal. Despite some improvements, current routines still risk predisposition to infection and failure or impose constant discomfort by placing heaviness on the top of the head.

New Methods. Using a custom-designed imaging adapter, MR and CT imaging of the head region were obtained. Then, based on reconstructed model of skull the implants were designed and constructed by CNC machine. During the surgical operation, the position of each implant was sketched on the skull and implants were slipped onto their predicted site and followed their sketched boundaries without any manual reshaping.

Results. We have performed this procedure on two monkeys. After surgery, location of implants has been verified by CT imaging. The recovery period was without significant complications with minimal infection.

Comparison with Exiting methods.

Our experiment showed that through the application of image-guided design, it is possible to better utilize the skull area to gain access to brain regions. At the same time, our method reduced the possibility of gap formation between implant and skull, open skin margins, and reduced the time and cost of operation which altogether results in a reduced overall chance of infection and failure and provides animal friendly operational surgery procedure.

Conclusion.

Despite some improvements, more refinements of methodology are still required. Here, we propose and report an improvement for the design and installation of biocompatible implants in low cost providing access to at least three brain regions.

Abbreviations: custom-made implant, electrophysiology, primate surgery

Introduction

Although long in use, extracellular single-unit recording in behaving animals is the technique still many studies draw upon to investigate temporal and spatial dimensions of cognitive activities. As a preparation step, it is required to install several chambers and a holding headpost on the skull. Chamber provides the interface for putting recording electrodes and drive system in place. Headpost or equivalent holding mechanism is used for stabilizing and protecting head motions. Along with this requirement comes a plethora of risks and considerations that are inherent in the invasiveness of the procedure. Post-operative acute and chronic infections, the possibility of chronic exposure of soft tissue due to incomplete closure of the skin after surgery, leading to an almost permanent infection risk. Furthermore, the chronic foreign body inflammations are the main complications and probable causes of implant failure.

While commercially available or custom-crafted versions of the implants are used for this purpose, these pieces need to be bent to fit onto their required position after inspecting the curvature. The headposts and chambers are either screwed on the bone or more commonly they are rooted into an acrylic base cover founded on a bolt and wire grid(Pfingst et al., 1989). The hand-bended headposts often results in leaving gaps between the implant and bone surface which increases the risk of infection and which also extends the period of surgery. Acrylic resins are used extensively as denture bases and bone cement. These products especially when leaking as monomers form from the polymer complex are known bioincompatible and toxic materials to the variety of tissues. Therefore, acrylic resins inhibit the successful regeneration of tissues after surgery. In addition, the heat produced during the application of the exothermic variants may injure contacting cells and resulting debris predisposes infecting bacterial growth(Jorge, Giampaolo, Machado, & Vergani, 2003). In recent years, there is a tendency to replace their usage with screw-only implants to reduce the risks associated with acrylic covers (Adams, Economides, Jocson, Parker, & Horton, 2011; X. Chen et al., 2017; McAndrew, VanGilder, Naufel, & Tillery, 2012; Mulliken et al., 2015).

Following magnetic resonance imaging (MR) and computerized tomography (CT) imaging, here we utilized a co-registered MR-CT reconstruction of the head region to make computer-aided design (CAD-designed) implants for two monkeys. Our suggested procedure applied the available skull areas and underneath brain structures to be within access of the recording chamber. This raises the availability of the target areas while reducing infection risks associated with an incomplete fit and longer surgical operations by providing an individualized design and a tight fit contact.

Here, we propose a protocol for custom crafting of the implants based on co-registered map of MR and CT series. We hope that this procedure has been easily adopted in neuroscience-based surgeries and be an improvement over some of the current methods and we also hope to increase the safety of the animals studied in neuroscience research.

Materials and Methods

Animals

Two adult male rhesus monkeys (*M. mulatta)* were used. All the procedures such as surgeries, post-operative care, behavior studies, and management conditions were strictly in accord with NIH guidelines for the care and use of laboratory animals and the internal regulations on animal care issued by the IPM-SCS committee for ethics. The entire process including the recovery phase and the time past before fixing the headpost for the first time took place in a 60-day interval.

CT and MRI imaging

For precisely co-registering different imaging modalities on the same coordination and having the same reference frame for surgery, it is necessary to use a CT and MRI compatible stereotaxic frame (TajhizGostar Iranian co, Iran) during imaging. In this adapter, the ear bars, the bite bar, and the eye pieces had to constrain the head in a manner exactly similar to a stereotaxic surgery frame. The proper fastening of the frame is essential and any misplacement produces errors in computations. Figure 1 depicts the reconstruction of the head and the constraining imaging adapter. We put several marker-filled capillary tubes filled with a contrasting lipophilic material (vitamin E) in the frame which makes it possible to set sliding steps for MRI image series in geometry. The marker-filled bars can be seen in rows both left and right and on the topside. Care should be given when positioning the head into the MRI (CT) bed. We performed the MRI (3.0 Tesla Siemens Prisma MRI Scanner) and CT (Somatom Spirit; Siemens) using this frame. It is possible to perform only a T2-Weight MRI without CT imaging. In this case, the contrast of the skull surface will decrease and more adjustments are needed when determining the threshold for reconstruction.

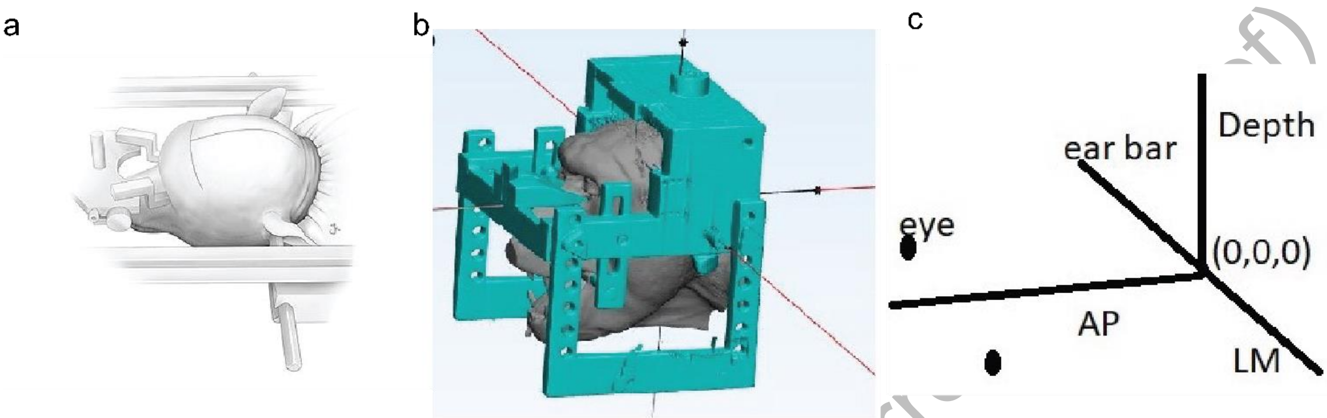

Figure 1. Similar frames for surgery and imaging based on monkey brain atlases, a) an animated of surgery stereotaxic, monkeys head is fixed by ear, eye and mouth bars. b) monkey head fasten in the imaging adaptor similar to surgery stereotaxic frame c) An animated 3D graph shoes coordination in both stereotaxic frames (the plane with depth=0 is the plane in which ear bar and eye pieces exist).

Preparing mesh model

We used the Materialise Innovation Suite research use version (Materialise Co, Belgium) to produce the initial 3d model of the skull and brain tissue. First, imaging series were segmented based on the alpha intensity band to isolate tissues of interest (bone/brain tissue).

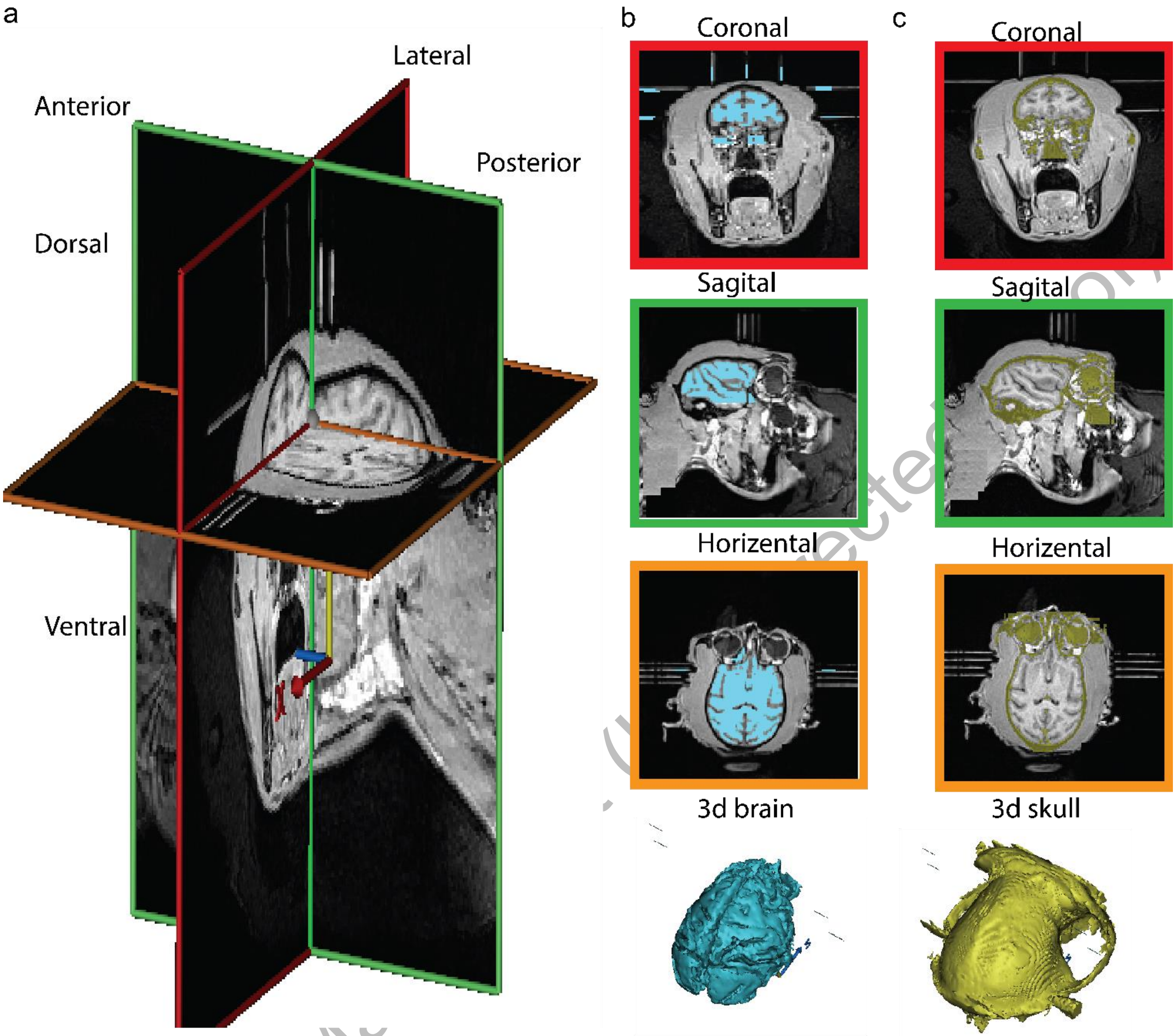

Figure 2. By image segmentation methods either brain and skull tissue extracted and 3D model of brain tissue and skull is produced in software, a) MR image series, were reconstructed in software. Markers of imaging adaptors is shown as white lines around beside the head of monkey. b) Different view of segmented brain tissue and also extracted the 3d model of brain in the bottom, c) similar to b for skull tissue.

Using different masks, we extracted skull and brain tissue separately. It might be necessary to edit the masks manually if the imaging contrast is not perfect in some regions of the images. After preparing the 3d model, we smoothed the skull surface to remove unwanted distortion (fig 3). It was needed to mesh the skull surface for CAD design. Mesh islands formed after threshold segmentation in the previous step can be removed through edge connection network and to remove the inner tablet of the skull, connection angle mesh extraction was used. Later, it was necessary to reduce mesh size and to smooth out distortions. Mesh size reduction makes working with mesh easier for lower-end processing units and smoothing distortions allows for better surface fitting when designing the implants (see next section). Quadratic reduction and Laplacian smoothing were used for mesh reduction and smoothing respectively(Z. Chen, Tristano, & Kwok, 2003). If necessary, we could apply manual mesh triangle removing and welding in some areas to prevent the rerunning of the smoothing algorithm over the already appropriate neighboring areas. It is reasonable to contain the mesh size and surface as close to the original reconstruction as possible since in either case, excessive change reduces the precision. In this stage, the boundaries of the areas of interest were extruded right to the skull surface to serve as guide during the design process. We targeted FEF and V4 in one monkey and PFC and IT in other monkey in this study. Then, the mesh was exported in the '.stp' format which allows for superior cross-platform compatibility between CAD software(Lanz et al., 2013).

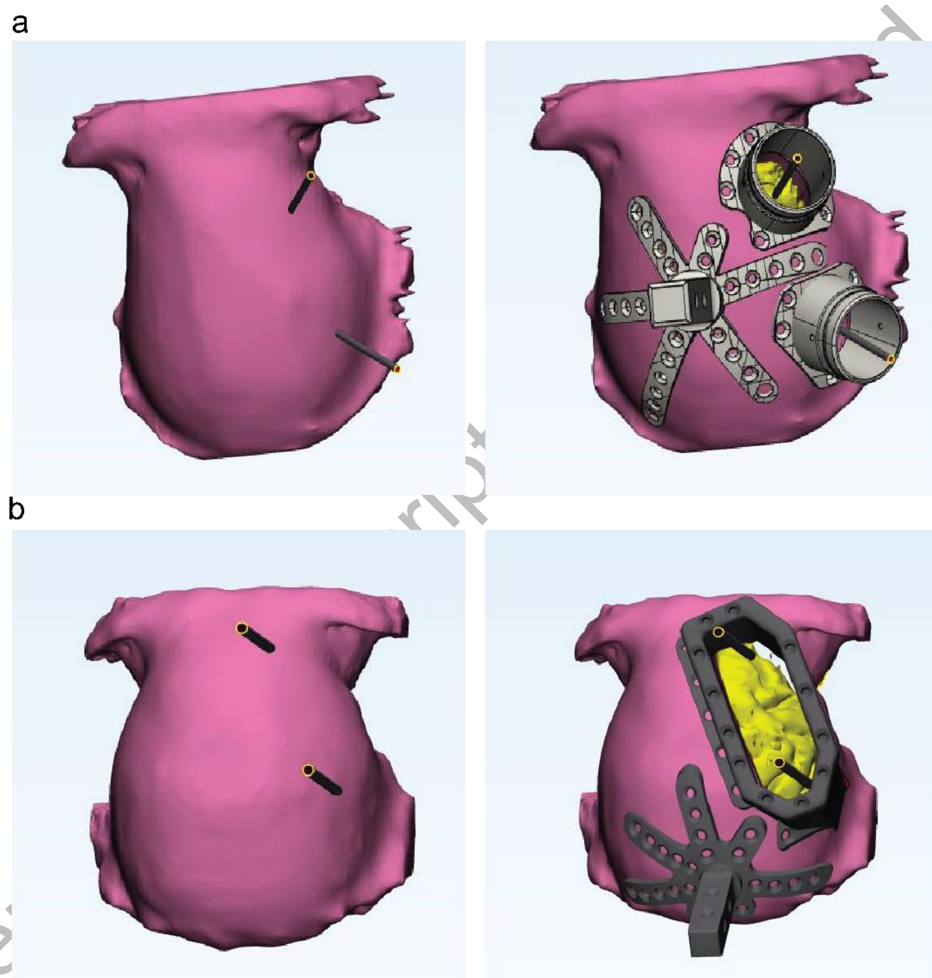

Figure 3. Reconstructed 3D model of the skull for both monkeys. The implants are CAD-designed considering their position relative to both the skull curvature. Monkey JK were used for FEF and V4 recording and monkey Z for PFC and IT recording. a) FEF located anterior to arcuate sulcus and V4 is anterior bank of the lunate sulcus b) For PFC and IT one chamber were used from lunate sulcus to anterior of principle sulcus.

Implant design and manufacturing

There are several good software options for implant CAD and the choice depends on the expertise, finance, or present access to the software. While computer graphics experts may find OpenGL as their solution, for the majority of researchers this is not an accessible option. An ideal solution must provide users with an integrated environment for the design and assembly of the parts and with tools to allow simulation of real geometry. Fortunately, at the moment, Autodesk provides a three-year free license for students and during this study we had access to the top-of-the-range Autodesk Inventor Pro 2018 (Autodesk Co, USA). We imported the prepared mesh model into Autodesk Inventor and a surface was fit over the relevant areas of the skull. Although it is possible to fit a single surface on the area of interest, sometimes especially when you plan to exhaust a large area or there is some problem with smoothing. It will be better to fit a separate surface for each implant. This takes some time but make sure to achieve a surface closely following the curvature of the model and do avoid extending the surface beyond the skull area overlying brain areas of interest. It works especially when you are aiming lower tolerance levels. Using this method, the fitting function works smoothly and does not try to achieve the coverage at the cost of reducing the precision (depending on the function definition and in the case that no exception is raised if you go beyond a certain limit). An axis through an arbitrary point on a vertex located preferably on the center of the skull mesh confined within the extruded brain area is used to define a plane perpendicular to the axis. The implants are sketched on such planes and are extruded to the fitted surfaces. After extruding all the implants, it becomes necessary to check if they are completely disjoint (if your design does not report or prevent geometry interference) and modify them if needed. The next step is defining the features of the implants. Sometimes achieving the exact dimensions requires local mesh manipulations which depending on your design platform, may result in overall design crash. In every step after such local manipulations check the entire implant mesh for any deviation from design. This is the most time-consuming part of the procedure and depends on the complexity of the part. The model of implants for one monkey exist in supplementary materials.

The implants were machined using a 3-axis computer numerical control (CNC) machine from medical grade titanium (Ti-6Al-4V ELI - Grade 23) in a single piece for each part (De Rezende & Johansson, 1993). To reduce the cost of fabrication, screw holes were created manually. This decision reduces the complexity of each part and the precision for the location of holes is in order of 1mm. To cut the costs, you can also 3d-print the skull model and craft aluminum implants to test the design before crafting the main titanium one's (figure 4)(X. Chen et al., 2017).

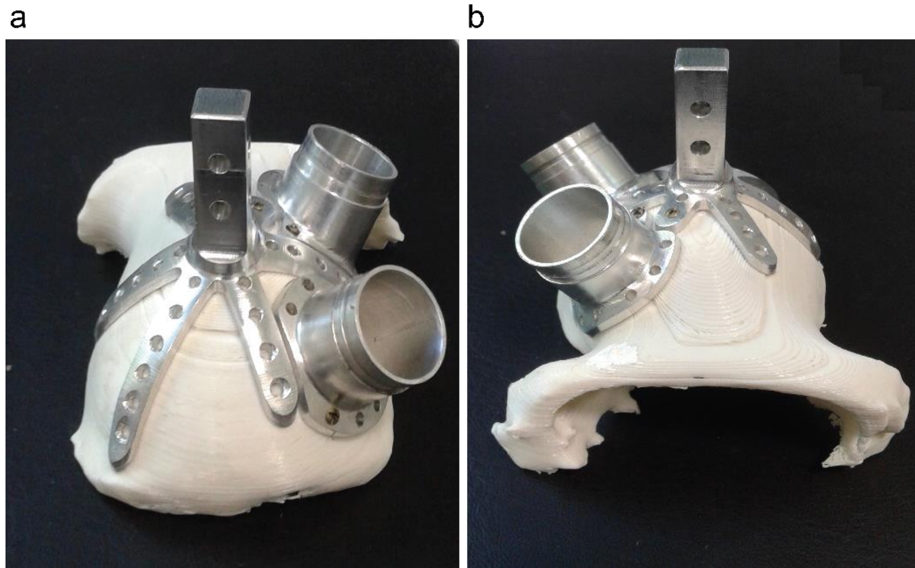

Figure 4. In one monkey, before construction of titanium implants, the model of aluminum implants and also 3D print model of skull in polylactic acid (PLA) materials were prepared. This figure shows implants were installed on skull's model, (a and b) posterior and anterior views respectively.

Surgery

The subject was anesthetized using diazepam and ketamine cocktail (7 mg/kg) for induction. For maintenance of anesthesia, the animal was intubated and ventilated with 0.8–1.5% isoflurane (20% O2and 80% air). The head region was placed and fixed in a stereotaxic frame (ToosBioResearch Co., Iran). On the eye and ear, tetracycline ointment was applied. Heart rate, SpO2, the temperature of the animal was checked during surgery(X. Chen et al., 2017). The skin of the skull was shaved and rinsed with 5% povidone-iodine scrub solution and the underlying bone was exposed by a coronal section across the midsagittal plane. Temporalis muscles on both sides were scraped off to make room for implants foot pieces. After cleaning the field with cotton swabs, the stereotaxic coordinates of each piece were identified and outlined with a surgical pen. The implants slipped to their places without any trouble and modification. A power drill with spotting drill bits (1 and 1.3 mm diameter) was used to mark entries. The self-drilling, self-tapping cortical titanium screws (2mm diameter and different length 5, 7, 9 mm; SYNTHES) were hand screwed in place muscle and skin flaps were restored to their original positions and stitched. Chamber's inner spaces were rinsed several times and a thin layer of cold-cure dental acrylic was applied within each chamber piece to prevent infection and save the underlying skull from necrosis before craniotomy. The craniotomy will be done in the next surgery. One day before and 2 weeks after surgery topical antibiotic were applied and the skin-implant margins were treated with topical tetracycline ointment. After two weeks no maintenance was performed and 50 days after the surgery, the headpost was fixed for the first time in the frame.

Result

One day after surgery the monkey regained its normal activity. The surgery inflammation disappeared in 5 days. The marginal gaps started to fill by fibrosis and all the areas were covered by fibrotic tissue after a month. There was no visible manifestation of infection at the site of surgery. There has been no sign of implants coming loose or any implication of infection or failure. We did not need to clean around the chamber and the headpost due to using biocompatible materials. This is the advantages of proposed method compare to the method which uses acrylic material for fixing headpost and chamber. One year after surgery, we performed a CT imaging. Figure 5 shows that the fibrotic tissue cores around the implants. One other observation was a significant reduction in surgery time (4-6 hours).

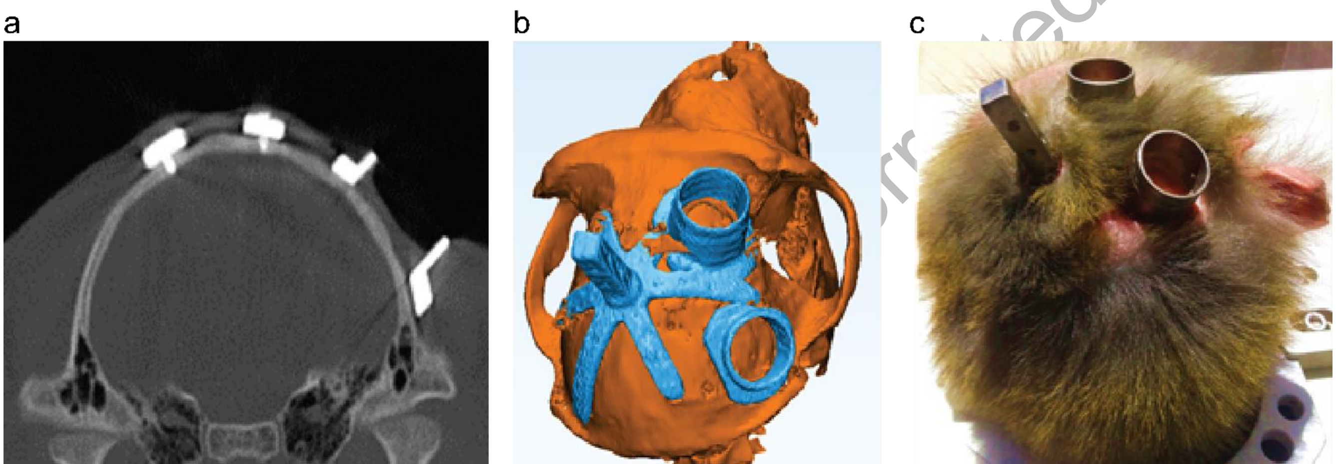

Figure 5. A CT scan imaging almost one year after surgery verified the location of implants a) Coronal section of CT it shows the depth of screw in the skull and coverage of skull tissue around the pedestals of headpost b) 3d model of skull with implants reconstructed based on CT scan image used to verification of the prearranged location of implants in models (c) One month after the operation the skin overs the implants were healthy and hairs raised around them.

Discussion

Regarding the considerable time and cost spent on preparing monkey subjects for behavioral neuroscience experiments, it's always a good idea to safeguard against probable sources of failure. Depending on the cognitive task, there is a long period before running recording experiment that monkeys are trained enough to participate in the actual task. As explained earlier implants used during recording studies may become sources of failure. Until recent decades the mostly applied protocol required the application of acrylic cement as a base for chambers and as a gap filler for both chambers and headposts (Betelak, Margiotti, Wohlford, & Suzuki, 2001). Many attempts, therefore, have been put to optimize each step, including the choice of material used, the shape of the implants and the method to fix them in place (Adams et al., 2011; McAndrew et al., 2012; Mulliken et al., 2015).

On the other hand, commercially available versions are generic and do not completely conform to the specific research requirements regarding the researcher's obsession with head motion or the ideal collocation of the implants for more efficient utilization of the skull area. Here we showed that it is possible to design and install implants that are only limited by the skull area. For instance, using our described method and to achieve better stability, we extended legs of the headpost both laterally to the temporal area and medially in a tangential manner in between the edges of the chambers. At the same time, we designed the chambers large enough to allow full access to the desired brain areas. The implemented parts held high fidelity to the design and literally slipped onto their predicted places. This is practically impossible, not mentioning its complications when you try to shape a nonspecific post or chamber during the surgery.

In two recent papers, custom-design approaches are proposed. In the first one(Mulliken et al., 2015), it is proposed to use specific PEEK materials for fabricating implants which our experience shows that these materials are not suitable for chronic usage especially for the headposts which receive large tension. The second study recommended titanium 3d printing which is an expensive method(X. Chen et al., 2017). Although, here we presented the procedure for implants used for typical single-unit recording studies, there is no limitation in extending its application to other similar or less similar studies or procedures where an implant is planned to be installed on the skull for animal studies or even for human surgical procedures.

Declaration of Competing Interest

The authors have no conflicts of interests to disclose.

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