Site-specific Implant design and installation for primate brain In vivo electrophysiological recording

Abstract

Introduction. During single unit recordings for cognitive studies on primates, it's necessary to install some implants on the skull. Despite some improvements, current routines still entail predisposition to infection and failure and require further development and refinement of methodology. Here, by building upon current improvements, we propose and implement still another improvement for the design and installation of such implants.
Methods. First CT and MR imaging of the head region was performed using the same imaging adapter for selecting the slicing steps and overall congruence of the imaged areas. The resulting series were registered into one coordinate system and a 3d-model of the skull and brain tissues was constructed. The position of the area of interest was selected and verified through collation with standard atlas and implants and their features including their curvature were CAD-designed according to the position of these areas and the overlying skull.
Results. During the surgical operation the position of each implant was sketched on the skull and implants slipped onto their predicted site and followed their sketched boundaries without any hand bending or other manual reshaping. Recovery period was without significant complications. Open margins at the boundary of skin and implants were minimal and the animal needed no daily maintenance except for the first few days after operation.
Conclusion. Our experiment showed that through application of Imaging guided design, it is possible to better utilize the skull area to gain access to brain areas. At the same time, our method reduced the possibility of gap formation between implant and skull, open skin margins, and reduced the time of operation which all together result in a reduced overall chance of infection and failure.

Abbreviations single unit recording, monkey electrophysiology, titanium implant, individualized implant

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 a number of chambers and a holding post on the skull. Chamber provides the interface for putting recording electrodes and their drive system in place and post or an equivalent holding mechanism is used for stabilizing and buffering head motions. Along with this requirement comes a plethora of risks and risk management considerations which 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, and chronic foreign body inflammation are the top complications and probable causes of implant failure .

For a typical study, It is common to have at least one chamber and a headpost implanted onto the skull. While commercially available or custom crafted versions are used for this purpose, these pieces need to be bent to fit onto their position after inspecting the curvature. The force bearing headposts are usually screwed fast into the underlying bone and chambers are either screwed likewise or more commonly they are rooted into an acrylic base cover founded on a bolt and wire grid (Pfingst *et al.*, 1989).

The on the go hand bending of the headpost foot piece 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 cements. These products especially when leak as monomer form from the polymer complex are known bio-incompatible and toxic materials to variety of tissues, they inhibit successful regeneration of tissues after surgery and the heat produced during application of the exothermic variants may injure contacting cells and the resulting debris predisposes bacterial growth and infection (Jorge *et al.*, 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 *et al.*, 2011; McAndrew *et al.*, 2012).

Here we utilize a co-registered MR-CT reconstruction of head region and computer-designed implants for one monkey and show its potential in better exhausting all the available skull areas and their underneath brain structures to be within access of the recording chamber. This rises the availability of the target areas while reducing infection risks associated with an incomplete fit and longer surgical operations through providing an individualized design and a tight fit contact. We try to be clear and to provide ample description of the process so that it will be readily replicable. In doing so, we hope others can easily find and adopt this method as an improvement over some of the current methods and we also hope it to increase the safety of the animals studied by these people.

Materials and Methods

Animals

One adult male rhesus monkey (*M. mulatta)* subject for an ongoing project at IPM School of Cognitive Sciences (SCS) was selected for the surgery. All the procedures coming below and the husbandry and management conditions were strictly in accord with with NIH guideline for the care and use of laboratory animals and the internal regulations on animal care issued by IPM-SCS committee for ethics. The entire process including the recovery phase and the time past before fixing the holding post for the first time, took place in a 60 day interval within February to April 2017.

Head region CT and MR Imaging

For co-registering the two series it is necessary to use an imaging adapter with contrasting lipophilic markers which makes possible to set sliding steps for MR and CT series. In this adapter, the ear-bars, bite bar and eye piece should constrain the head in a manner like standard streotaxic frames. The proper fastening of the frame is essential and any misplacement produces errors in computations. Figure 1-a depicts the reconstruction of the head and the constraining Imaging adapter. The marker-filled bars can be seen in rows both left and right and on topside. The same frame is used when taking MR series. Care should be given when positioning the head into the MR or CT bed. We devised a circular level and leveling legs for this purpose .

Preparing mesh model

There are both open source and commercial scripts or applications for 3d reconstruction. For this study we used Materialise Innovation Suite (Materialise Co, USA) to produce the initial mesh and its preparation for CAD design as described in the following. First, imaging series were segmented based on alpha intensity band to isolate tissues of interest. Mesh islands formed after threshold segmentation in the previous step can be removed through edge connection network mesh extraction and connection angle mesh extraction is used to remove the inner tablet of the skull. Later, It is 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. If necessary you can apply manual mesh triangle removing and welding in some areas and this way prevent rerunning of the smoothing algorithm over 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 location of the area of interest was extruded right to the skull surface to serve as a guide during design process and the mesh was exported in STEP format, which allows for superior cross-platform compatibility between CAD softwares.

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 expert may find OpenGL as their solution, for majority of researchers this is not an accessible option. An ideal solution must provide users with an integral environment for design and assembly of the parts and with tools to allow simulation of real geometry. During this study we had access to the top-of-the-range Autodesk Inventor Pro 2016 (Autodesk Co, USA) but as we know almost the same procedure is applicable for the users of other softwares such as rhinoceros and Solidworks and in each software case there are alternative ways to achieve the same goal as long as basic engineering design principles are taken into account. 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, some times especially when the 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 especially when you are aiming lower tolerance levels, this way 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 program does not report or prevent geometry interference) and modify them if needed. Next step is defining the features of the implants. Some times 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 implants were machined using a 3-axis CNC machine from medical grade titanium in single piece. To reduce the cost of fabrication, screw holes were created manually. this decision reduces the complexity of each part and the precision for 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 ones.

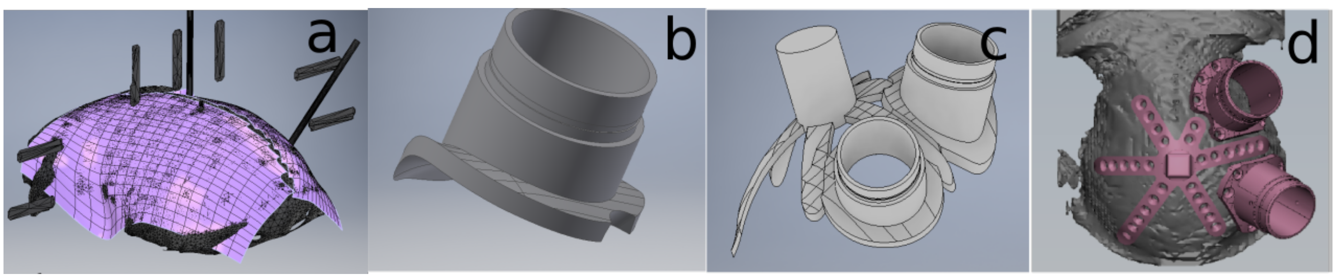

Figure 2. a) a surface is fitted on the reconstructed 3d model of the skull; (b and c) implants are CAD-designed considering their position relative to both the skull curvature and themselves; (d) designs are finalized.

Surgery

Subject was anesthetized using diazepam + ketamine cocktail for induction and Isoflurane inhalation for maintenance. The head region was placed and fixed in a stereotaxic frame (ToosBioResearch Co., Iran). The scalp was shaved and rinsed with 5% povidone iodine scrub solution and the underlying bone was exposed by a coronal section along midsagittal plane. Temporalis muscles on both sides were scraped off in order 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 surgical pen. The implants slipped to their places without any trouble and modification (figure 3-a). A power drill with spotting drill bits was used to mark entries and self drilling, self-tapping cortical titanium screws were hand screwed in place under an approximate torque of 20 Nm. the muscle and scalp flaps were restored to their original positions and stitched(figures 3-b and 3-c). Chambers 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.

For four days following surgery the animal was restrained in cage and received prophylactic … and … intramuscularly three times a day. Once a day for four days, the monkey was sedated and the skin implant margins were pressure rinsed with normal saline solution. On the fifth day and for the following three weeks, while conditioning the subject for sitting in chair, and when the subject was restrained in the chair with his head free, the skin-implant margins was treated with topical tetracycline ointment. After 50 days after surgery the headpost was fixed for the first time in the frame.

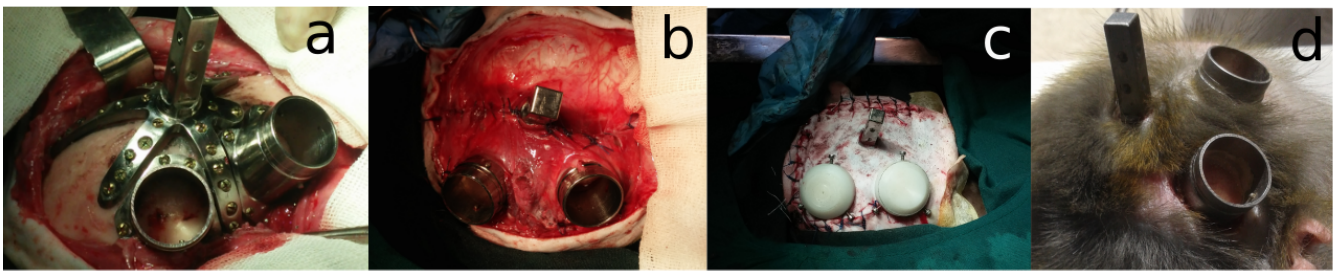

Figure 3. a) skull is exposed and implants are placed as predicted. They slipped into their predicted geometry with no need in ; (b) connective tissue is back in place covering all the implant attachments; (c) skin is stretched over, and cut to fit around the implants; (d) one month after the operation.

Results

One day after surgery the monkey regained Its normal activity. The surgery inflammation disappeared in 5 days and within 4 days, the marginal gaps started to fill by fibrosis and after a month, all the areas were covered by fibrotic tissue. There was no visible manifestation of infection at the site of surgery and after the first few days after the operation, the animal needed no daily toilet and was on track to learn the task. From the day 50 to the time of writing this paper (about 7 months), there has been no sign of implants coming loose or any implication of infection or failure. One other observation was significant reduction of surgery time (to a fifth) compared to the current routines at SCS based on the existing histories.

Discussion

Regarding the considerable time and cost spent on preparing monkey subjects for cognitive experiments, it's always a good idea to safeguard against probable sources of failure. In North America, for example, the costs for such preparation in case of a rhesus monkey (including) and for a single unit experiment in a traditional headpost-chamber setting, fall well in range of 50000 to 70000 USD (acquired by auditing colleague labs). Apart from that, there is a time spanning several months at best depending on the cognitive task at hand, before monkey subjects are trained enough to participate in the actual experiment. As explained earlier implants used during recording studies may become sources of failure and until recent decade the only available protocol required application of acrylic cements as a base for chambers and as a gap filler for both chambers and headposts (Betelak *et al.*, 2001) whose associated complications was shown before. many attempts therefore have been put to optimize each step, including choice of material used, shape of the implants and the method to fix them in place (Adams *et al.*, 2007, 2011; McAndrew *et al.*, 2012; Mulliken *et al.*, 2015).

On the other hand, commercially available versions like CRISTA ... are generic and do not conform to the specific research requirements regarding the researcher obsession with head motion or the ideal juxtaposition of the implants for more efficient utilization of the skull area. Here we showed that it is possible 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 holding post both laterally to the temporal area and medially in a tangential manner in between the edges of the chambers to the opposite temporal area with relative ease and at the same time 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 place. This is practically impossible, not mentioning its complications, when you try to shape a nonspecific post or chamber during the surgery.

Although in 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.

References

Adams, D. L. *et al.* (2007) ‘A biocompatible titanium headpost for stabilizing behaving monkeys’, *Journal of neurophysiology*. Am Physiological Soc, 98(2), pp. 993–1001.

Adams, D. L. *et al.* (2011) ‘A watertight acrylic-free titanium recording chamber for electrophysiology in behaving monkeys’, *Journal of neurophysiology*. Am Physiological Soc, 106(3), pp. 1581–1590.

Betelak, K. F. *et al.* (2001) ‘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*. Elsevier, 112(1), pp. 9–20.

Jorge, J. H. *et al.* (2003) ‘Cytotoxicity of denture base acrylic resins: a literature review’, *The Journal of prosthetic dentistry*. Elsevier, 90(2), pp. 190–193.

McAndrew, R. M. *et al.* (2012) ‘Individualized recording chambers for non-human primate neurophysiology’, *Journal of neuroscience methods*. Elsevier, 207(1), pp. 86–90.

Mulliken, G. H. *et al.* (2015) ‘Custom-fit radiolucent cranial implants for neurophysiological recording and stimulation’, *Journal of neuroscience methods*. Elsevier, 241, pp. 146–154.

Pfingst, B. E. *et al.* (1989) ‘Chronic skull-anchored percutaneous implants in non-human primates’, *Journal of neuroscience methods*. Elsevier, 29(3), pp. 207–216.