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MRI-Based Toolbox for Neurosurgical Planning in Non-Human Primates

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Abstract

In this paper, we outline a method for surgical preparation that allows for the practical planning of a variety of neurosurgeries in NHPs solely using data extracted from magnetic resonance imaging (MRI). This protocol allows for the generation of 3D printed anatomically accurate physical models of the brain and skull, as well as an agarose gel model of the brain modeling some of the mechanical properties of the brain. These models can be extracted from MRI using brain extraction software for the model of the brain, and custom code for the model of the skull. The preparation protocol takes advantage of state-of-the-art 3D printing technology to make interfacing brains, skulls, and molds for gel brain models. The skull and brain models can be used to visualize brain tissue inside the skull with the addition of a craniotomy in the custom code, allowing for better preparation for surgeries directly involving the brain. The applications of these methods are designed for surgeries involved in neurological stimulation and recording as well as injection, but the versatility of the system allows for future expansion of the protocol, extraction techniques, and models to a wider scope of surgeries.

SUMMARY:

The method outlined below aims to provide a comprehensive protocol for the preparation of non-human primate (NHP) neurosurgery using a novel combination of three-dimensional (3D) printing methods and MRI data extraction.

Keywords

non-human primates; magnetic resonance imaging; neurosurgical planning; 3D printing; viral vector delivery; optogenetics

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INTRODUCTION:

Primate research has been a pivotal step in the progression of medical research from animal models to human trials[1,2]. This is especially so in the study of neuroscience and neural engineering as there is a large physiological and anatomical discrepancy between rodent brains and those of non-human primates (NHP)[1–3]. With emerging genetic technologies such as chemogenetics, optogenetics, and calcium imaging that require genetic modification of neurons, neural engineering research studying neural function in NHP's has gained special attention as a preclinical model for understanding brain function[2,4–16]. In most NHP neuroscience experiments, neurosurgical measures are required for the implantation of various devices such as head posts, stimulation and recording chambers, electrode arrays and optical windows[4–7,10,11,13–15,17,18].

Current NHP labs use a variety of methods that often include ineffective practices including sedating the animal to fit the legs of a head post and approximate the curvature of the skull around the craniotomy site. Other labs fit the head post to the skull in surgery or employ more advanced methods of gaining the necessary measurements for implantation like analyzing an NHP brain atlas and magnetic resonance (MR) scans to try to estimate skull curvatures[2,10,11,16]. Neurosurgeries in NHPs also involve fluid injections, and labs often have no way to visualize the projected injection location within the brain[2,4,5,13,14] relying solely on stereotaxic measurements and comparison to MR scans. These methods have a degree of unavoidable uncertainty from not being able to test the physical compatibility of all the complex components of the implant.

Therefore, there is a need for an accurate non-invasive method for neurosurgical planning in NHPs. Here, we present a protocol and methodology for the preparation of implantation and injection surgeries in these animals. The whole process stems from MRI scans, where the brain and skull are extracted from the data to create three dimensional (3D) models that can then be 3D printed. The skull and brain models can be combined to prepare for craniotomy surgeries as well as head posts with an increased level of accuracy. The brain model can also be used to create a mold for the casting of an anatomically accurate gel model of the brain. The gel brain alone and in combination with an extracted skull can be used to prepare for a variety of injection surgeries. Below we will describe each of the steps required for the MRI based toolbox for neurosurgical preparation.

PROTOCOL:

All animal procedures were approved by the University of Washington Institute for Animal Care and Use Committee. Two male rhesus macaques (monkey H: 14.9 kg and 7 years old, monkey L: 14.8 kg and 6 years old) were used.

1. Image acquisition

1.1. Transport the monkey to a 3T MRI scanner and place the animal in an MR-compatible stereotaxic frame (Table of Materials).

1.2. Record standard T1 (flip angle = 8°, repetition time/echo time = 7.5/3.69 s, matrix size = 432 × 432 × 80, acquisition duration = 103.7 s, Multicoil (Table of Materials), number of averages = 1, slice thickness = 1 mm) anatomical MR images.

NOTE: For successful skull isolation, use the MRI acquisition parameters applied here to maximize separation between skull and brain.

2. Brain extraction

2.1. In the MR imaging software for brain extraction select ‘Open’, ‘Open Image’, and load the T1 Quick Magnetization Prepared Rapid Gradient Echo (MPRAGE) scan acquired in step 1.2 into an MR imaging software (Table of Materials).

2.2. To extract the brain, under the “Plugins” drop down menu select “Extract Brain (BET)” and extract at an intensity threshold around 0.5− 0.7 and set the threshold gradient value to 0. Repeatedly use the extraction function at successively lower intensity thresholds until the scan contains only the cortical anatomy (Figure 1B).

NOTE: This is an iterative process because the software is not designed for NHP brains and extraction is not precise

2.3. Under the region of interest (ROI) menu select ‘Threshold to ROI’ and select the option for ‘Shrink Wrap’ and ‘3D’ in order to create a bitmap of the brain. This will convert the volume to binary bits from a gradient, which streamlines future model generation processes. Choose a threshold (usually around 600) to isolate the brain from the surrounding tissue. This threshold can be found by hovering over the gray matter. Select ‘ok’ to form the bitmap.

2.4. To create a surface, select ‘build surface’ under the image menu and input the threshold used to extract the brain in step 2.3 then select ‘ok’. The resulting surface can be used as a reference for adjusting the threshold value to produce the highest quality representation of the targeted anatomy (Figure 1C).

2.5. Under the file tab select ‘save’ or ‘save as’, and save the extracted brain ROI as a .nii or .nii.gz file for use in creating the model of the brain.

3. Brain modeling

3.1. Select ‘Load Data’ and then ‘Choose Files to Add’ and load the extracted brain saved in the .nii or .nii.gz file type in medical image processing software (Table of Materials).

3.2. Hover over the welcome to slicer drop down menu in the modules toolbar and move to all modules. From that menu select the ‘editor’ functionality. Click ‘ok’ on the popup warning.

3.3. From the ‘editor’ module menu, select ‘Threshold Effect’ and adjust the threshold range sliders so the portion of the bitmap containing the brain is highlighted in all three slices. When loading a bitmap, adjusting both sliders to a value of 1 selects the whole brain. Select ‘apply’.

3.4. Open the model maker module, and in the input volumes dropdown menu select the bitmap file generated in 3.3. Under ‘models’ select ‘create new model hierarchy’. After assigning the name of the model to the hierarchy, select ‘apply’ to create the volume.

3.5. Save the file in the .stl format.

3.6. To further modify the brain model, load the .stl file as a solid body in computer aided design software (Table of Materials)

NOTE: This may take a while, as the imported mesh brain surfaces are often quite complex.

3.7. Once the file is imported, in the feature tree on the left side of the screen click on the rollback bar and drag it up to remove any floating solid bodies until only the features containing the brain are remaining in the file. Save the remaining file as a .prt for further manipulation and as a .stl for 3D printing.

4. Brain molding

4.1. Load the extracted brain model from section 3 to the computer aided design software by opening the .prt file.

4.2. Creating a right and left mold of the full brain

4.2.1. Click the sketch button and select the top plane as the sketch plane. Draw a rectangle containing either the entirety of the right or left hemisphere of the brain. Select the extrude boss/base feature while in the sketch and extrude a cubic rectangle to contain the top part of the brain.

NOTE: The cube may have to be extruded in two directions in order to contain the entire hemisphere. This is because the zero point, where the plane of extrusion is located, may fall inside the brain model. Extruding in both directions ensures that the mold will encompass the whole volume of interest.

4.2.2. To create the negative space, subtract the model of the brain from the newly extruded cube using the “combine” feature and selecting the subtract option.

4.2.3. Repeat steps 4.2.1 and 4.2.2 for the other hemisphere of the brain (left or right) and save the resulting files as a .stl for 3D printing and a .prt for further manipulation.

4.3. Creating a right and left mold of the upper half of the brain.

4.3.1. Create a sketch in the top plane and draw a rectangle containing either the entirety of the right or left hemisphere of the brain. Select the extrude boss/base feature while in the sketch and extrude with the “offset from plane” feature selected. Offset the extrusion up to a distance where there are no overhanging contours in the brain anatomy, capturing just the upper anatomy.

4.3.2. To create the negative space, subtract the model of the brain from the newly extruded cube using the “combine” feature and selecting the subtract option.

4.3.3. Create a sketch plane on the dorsal side of the mold and select “convert entities” and then select the sketch from step 4.3.1.

4.3.4. Select the extrude boss/base feature while in the sketch and, with the blind extrude option selected, extrude the solid body approximately 5 mm to completely enclose the subtracted brain anatomy in the mold.

4.3.5. Repeat steps 4.3.1−4.3.4 for the other hemisphere of the brain (left or right) and save the resulting files as a .stl for 3D printing and a .prt for further manipulation.

4.4. By changing the dimensions and location of the cube and following the same protocol (steps 4.1 and 4.2), create molds that contain different parts of the brain.

4.5. For 3D printing, use ~70% infill density and increase the thickness of the outer shell of the print in order to minimize leakage of the molding material. If there are gaps or defects in the print, fill them using nail polish or another binding agent.

5. Skull modeling

5.1. Import QUICK MPRAGE MRI from step 1.2 into matrix manipulation software as a DICOM file. The DICOM file may be in separate 2D frames. If this is the case, combine all frames into a 3D matrix. Ensure that each 2D frame of the matrix is displaying a coronal slice.

5.2. Create a binary mask by thresholding the 3D matrix using a greater than operator for individual pixel values. Adjust the threshold such that the skull anatomy is being captured by the mask (see Supplemental coding file “CalibrateMask”).

NOTE: The mask will contain four distinct layers. From the outside in, they will be referred to as “outside”, “musculature”, ”skull”, and “brain”. At this stage, “outside” and “skull” are 0’s in the mask, and “musculature” and “brain” are 1’s.

5.3. To remove the “musculature” layer, process each frame from the 3D mask separately by iteratively grabbing a 2D slice from the mask (i.e. 3D_Mask(:,:,1)). For each frame, select 0 pixels from the corners of the frame in the “outside” layer as the “seed”. Then search neighboring 0’s until you encounter a 1 pixel. Continue searching until no more 0’s can be found. Convert all connected 0’s to 1’s . This is done using the matlab function “imfill”, with the inputs and outputs being [MASK2] = imfill(MASK1,LOCATIONS,CONNECTIVITY), where MASK1 is your original mask, and MASK2 is the filled in mask (see Supplemental coding file “FillExterior”).

5.4. Some skull information will be lost during the removal. To mitigate the information loss, perform step 5.3 in all three dimensions of your data, and keep them separate.

NOTE: Now both “outside” and “musculature” are 1’s, and will be considered as “outside”. The mask now contains three distinct layers, “outside”, “skull”, and “brain”. “Outside” and “brain” are 1’s, and “skull” is 0’s.

5.5. Invert the values of the mask using the ~ operator (i.e. MASK2 = ~MASK1). Now "skull" is 1's and "outside" and "brain" are 0's.

5.6. The 1's that are touching each other in each mask can be considered "objects". Create an index of all objects in each mask using the Matlab function "bwconncomp", with the inputs and outputs being [CC] = bwconncomp(MASK), where MASK is your 3D mask matrix, and CC is a structural object containing the index values of for each object, the number of objects and the size of the matrix. For each mask, remove all objects except for the largest one containing the most voxels by setting the values of the smaller objects to 0's (see Supplemental coding file "RemoveNoise").

5.7. Add the masks created from each pass together (see Supplemental coding file "MergeMasks").

5.8. Scale the brain to a consistent resolution.

5.8.1. From the DICOM header, compare the step size between each frame of the MRI to the dimensions of each pixel.

5.8.2. If these values are different, define a scale factor to compensate for the difference in resolution between step size and pixel size for each voxel. For example, if each frame is 1 mm apart, and the pixel dimension is 0.33 mm x 0.33 mm, the scale factor will be 3.

5.8.3. Add additional empty voxels to the 3D mask until the lowest resolution dimension of the mask is larger by a factor defined by the scale factor (see Supplemental coding file "ScaleMask").

5.8.4. Linearly interpolate values in the mask until the mask fills the new space.

5.8.5. Export skull as an .stl file or similar filetype for 3D printing.

6. Craniotomy creation in the 3D skull model

6.1. Using the MRI file from step 5.1, manually identify the approximate location of the craniotomy from anatomical landmarks found in the macaque brain atlas (e.g. central sulcus)[19].

6.1.1. View an individual slice of the 3D matrix (Similar to step 5.3).

6.1.2. Manually scan forward or backward through the 3D matrix until recognizable anatomical landmarks are located.

6.1.3. Save the frame number as your z coordinate (i.e. 3D_Mask(:,:,z)

6.1.4. Use a data selection tool to save the x and y coordinates of a single point on this frame for the craniotomy to be centered on using the Matlab function "getpts", with the inputs and outputs being [x,y] = getpts. "getpts" opens up a user interface, click on the desired frame (see Supplemental coding file "LocateCraniotomy").

6.2. Convert the radius of the intended craniotomy from mm to voxels using the information in the DICOM header.

6.3. Using the point specified in step 6.1 as a centerpoint, set all voxels within the radius defined in 6.2 to zero in the mask from step 5.8.4 using Supplemental coding file "Craniotomy", where the inputs and outputs are [craniotomyMask] = Craniotomy(mask, x, y, z, radius, X, Y, Z, resolution)where cranitomyMask is a 3D matrix with a the craniotomy removed, mask is the initial 3D matrix, x,y,z are the centerpoint coordinates of the craniotomy, radius is the radius of the craniotomy, X,Y,Z are grid vectors of the 3D matrix, and resolution is your radius defined in step 6.2 (see Supplemental coding file "Craniotomy").

6.4. For multiple craniotomies, repeat steps 6.1−6.3 for each unique craniotomy.

6.5. Export skull as an .stl file or similar filetype for 3D printing.

7. 3D printing—NOTE: Two types of 3D printers for physical prototypes (Table of Materials) are used. For the following specifications, all 3D printer and printing software settings should be default unless otherwise mentioned.

7.1. In order to print the prototypes and molds, use a standard PLA printer (Table of Materials) and create the G-Code with the following printer and software settings: inner density >50% (this is especially important for the molds as they must hold liquid), fast honeycomb internal fill pattern, rectilinear external fill pattern, plate temperature = 50 °C, and extruder temperature = 230 °C.

7.2. In order to print higher fidelity models of the brain and skull use an industrial-grade printer to make a combined print of Acrylonitrile Butadiene Styrene (ABS) for the model and a dissolvable support material (Table of Materials). Then create the G-Code and with the following printer settings: fill style Sparse - High Density, all other settings will be automatically set to the appropriate default setting, and the model must be dissolved in support solvent (Table of Materials) for ~12 h.

7.3. After implementing the appropriate printer settings, press start and watch the first layer of the print to ensure that the base layer is clean and even.

7.4. After 3D printing the molds, patch any visible holes with nail polish (Table of Materials) to guarantee a tighter seal.

NOTE: The 3D printed brain and skull models can be combined by inserting the brain model into the open bottom of the skull. Removing the eye anatomy can ease the placement of the brain model without losing important information. When placed inside the skull, the brain naturally aligns to the anatomically correct position.

8. Preparation of agarose

8.1. Mix the agar powder (Table of Materials) and locust bean gum powder (Table of Materials) in a 1:4 ratio by mass.

8.2. Combine the powder mixture with 1x phosphate buffered solution (Table of Materials) to a 0.6% concentration solution in an Erlenmeyer flask.

NOTE: Concentrations used by other labs falls in a range of 0.5%−0.6%[20,21] .

8.3. Set the microwave to max power and place the flask containing the solution in the microwave for 2 min.

8.4. Observe the solution. When the solution begins to bubble, stop the microwave and timer, remove the flask, and swirl vigorously. Set the flask back in microwave and resume the microwave and timer.

NOTE: The purpose is to heat the solution without reducing the volume significantly due to evaporation during boiling.

8.5. Repeat step 8.4 until the two minutes is complete.

8.6. Remove the flask and maintain swirling to prevent the solution from setting in the flask.

8.7. Run cold water on the outside of the flask to cool the solution while swirling. Cool the solution until the outside of the flask is hot to the touch, yet tolerable and safe, to prevent the solution from deforming the plastic mold in the following steps.

8.8. Swirl the flask while transporting the solution to the mold to avoid premature hardening.

9. Agarose molding

NOTE: The agarose molding process outlined below is the same for the full hemisphere and upper half hemisphere molds

9.1. Pour the agarose solution into one of the brain molds until full. Continue to swirl remaining solution in the flask.

9.2. Monitor the level of solution in the mold for leaks. Refill the mold as necessary since the setting agarose will seal any leaks in the mold.

9.3. Allow the solution in the mold to sit unperturbed at room temperature until the solution has set and hardened into a solid.

NOTE: While wait times may vary depending on the volume of solution and other factors, 2 h is found to be a reliable wait time.

9.4. Use a spatula to gently remove the gel model from the mold. Be strategic with the location of spatula insertion into the mold in order to prevent potential deformations to the surface of the mold.

9.5. To slow the natural evaporation process and exposure to biological agents, place the gel model in a sealed container in a refrigerator.

10. Injection into agarose gel model

10.1. Prepare the pump for infusion and fix it in a stereotaxic arm on a stereotaxic frame (Table of Materials). Position the pump to the correct injection trajectory and location normal to the surface of the gel model from section 9.

10.2. Fill 250 μL syringe (Table of Materials) with DI water. Mount the syringe onto the pump (Table of Materials).

NOTE: Before drawing any dye, DI water should fill the injection cannula (Table of Materials) completely. That way when the dye is drawn up through the cannula there is no compression or expansion of air by the plunger that might impact the injection volume.

10.3. Use the pump driver (Table of Materials) to withdraw the food coloring (Table of Materials) into the syringe to the target volume for injection. Inject the food coloring slowly until a small bead forms at the tip of the cannula to prevent air bubbles from being injected into the gel. Dry the bead off the tip of the cannula.

10.4. Position the gel model under the cannula. Lower the cannula until the tip touches the surface of the gel model. Note the measurements on the stereotaxic arm.

10.5. Lower the cannula into the gel model quickly and smoothly to the target injection depth and ensure that the surface of the gel has sealed around the cannula.

10.6. Run the pump and observe the spread of the food coloring until the target volume is injected. Our flowrate started with 1 μL/min and was increased to 5 μL/min with 1 μL/min steps every minute. The spread of the food coloring in the gel is an approximation of the spread of a viral vector in the brain.

10.7. Remove the cannula from the gel quickly and smoothly.

10.8. Capture images of the spread of the food coloring with a photographic device (see Table of Materials)and physically measure the dimensions of the embolism in order to calculate the ellipsoid volume of the injection. This approach is possible due to transparent nature of the gel.

REPRESENTATIVE RESULTS:

The manipulation and analysis of MRIs as a preoperative craniotomy planning measure has been used successfully in the past[2,5,10,16]. This process, however, has been greatly enhanced by the addition of our 3D modeling of the brain, skull, and craniotomy. We were able to successfully create an anatomically accurate physical model of the brain that reflected the area of interest for our studies (Figure 1). We were similarly able to create an anatomically accurate physical model of the primate skull extracted from the MR images (Figure 2).

The two physical models of the skull and brain combined with a tight interference fit, validating the accuracy of the two models relative to each other and legitimizing the extrapolated MRI analysis data (Figure 3A,B). With the combined model we were able to insert a craniotomy into the skull prior to printing and visualize the predicted anatomy

in the craniotomy (Figure 3). The accuracy of the predicted anatomy in the craniotomy was validated through a comparison of the physical model and the predicted craniotomy from MRI analysis (Figure 3B). Additionally, we were able to combine all of the parts of our example interface and evaluate the geometry of the various components in relation to the skull and brain (Figure 3C,D).

In order to test the skull model, a physical model of the skull of Monkey L was extracted using the methods outlined above and 3D printed to plan for a head post implantation surgery. The feet of the head post were then manipulated and fitted to the curvature of the skull at the location of the implantation (Figure 3E). As a result of the preoperative fitting of the head post, the surgery time was reduced from approximately 2.5 hours to 1 hour (216% faster) from opening to implantation, greatly reducing the risk of operative complications[22].

By manipulating the 3D model of the brain in SolidWorks, we were able to create a mold that accurately reflected the anatomy of both the printed brain and the brain model extracted from the MRI (Figure 4A−C). This mold was used to cast an agarose mixture model of the brain (Figure 4D,E). Using these molds of the brain, we were able to inject in different areas of the brain and estimate the volume of the infusion of an injection procedure modeled with a yellow dye (Table of Materials). The half-hemisphere gel model of the brain was successfully used to capture a clear view of the spread of the dye in a model virus injection, allowing us to measure an approximate volume of the dye over time as it was injected (Figure 5A). Injection of dye into the brain model was combined with a 3D printed skull to model viral vector injection surgery (Figure 5B,C). This was combined with an electrocorticography array placement on top of the injection to guide the implantation in preparation for surgery[7,10].

DISCUSSION:

This article describes a toolbox for preparation for neurosurgeries in NHPs using physical and CAD models of skull and brain anatomy extracted from MR scans.

While the extracted and 3D printed skull and brain models were designed specifically for the preparation of craniotomy surgeries and head post implantations, the methodology lends itself to several other applications. As described before, the physical model of the skull allows for pre-bending of the head post before surgery, which creates a good fit with the skull. Moreover, the extracted skull from MRI can be used to generate a 3D designed head post with a higher fidelity to the skull anatomy. While CT imaging is traditionally a better modality for skull extraction, in our proposed method, the brain and skull anatomy come from the same imaging modality, which could contribute to enhanced anatomical consistency between the bone and soft-tissue models. This anatomical consistency could enhance precision and ensure that the craniotomy will cover the cortical region of interest and that all the implanting components, such as stimulation and recording chambers, fit the skull curvature. This is supported by extant studies that quantitatively compared MRI-extracted skull topography to extractions from other scan types[23,24]. Other work in the field has outlined methods for the creation of models and 3D printed prototypes for head post implantation[25,26], but they do not use solely MR scans to create an adaptable model

for the preparation for both head posting and craniotomy. It is important to note that the MRI acquisition parameters used here are critical in successful skull extraction as outlined in the protocol. Previous work in the field of brain extraction and skull stripping offers alternatives to the widely available BET brain extraction used in this protocol[27]. Similarly, skull extraction custom scripts exist, however, they require the manual removal of non-skull voxels compared to our completely automated protocol[28]. While here we show only a few examples, these tools are applicable to a variety of other surgeries such as electrode and chamber implantations in NHPs[2,4,5,7,10,15,18,29,30], as well as other animal models[31,32].

When combined with the agarose mixture brain models, the surgical preparation toolbox can be applied to prepare for surgical procedures involving fluid injections such as optogenetics and chemogenetics[2,4,5,10,33,34]. Although here we had success with using PLA to 3D print the molds, this process can be further improved by using an ABS filament, which has a higher glass transition temperature that will make the molding process more efficient. Prior work has proposed agarose gel as an artificial material that can mimic some of the mechanical properties of the brain relevant to fluid infusion[20,21]. However, previous work has not combined the agarose with a brain-realistic mold to provide a surgical preparation tool. The molded agarose mixture gel brains can be used to give qualitative cortical context to the injection location and visualize the volume and location of fluid diffusion. The gel brains can also be used to practice the injection motion and location within the stereotaxic frame. This can be applied not only to optogenetics but translated into other experiments requiring injection into the brain[2,4,34]. The model can also be used to enhance the current CED standard practice by optimizing injection speed and cannula thickness. This model can also be strengthened by the quantitative validation of the agarose gel mixture to accurately represent diffusive and convective flow in the brain[5,10]. In future efforts we can also incorporate vasculature information into our 3D models by including contrast-enhanced imaging to our imaging procedure which can provide critical information on injection planning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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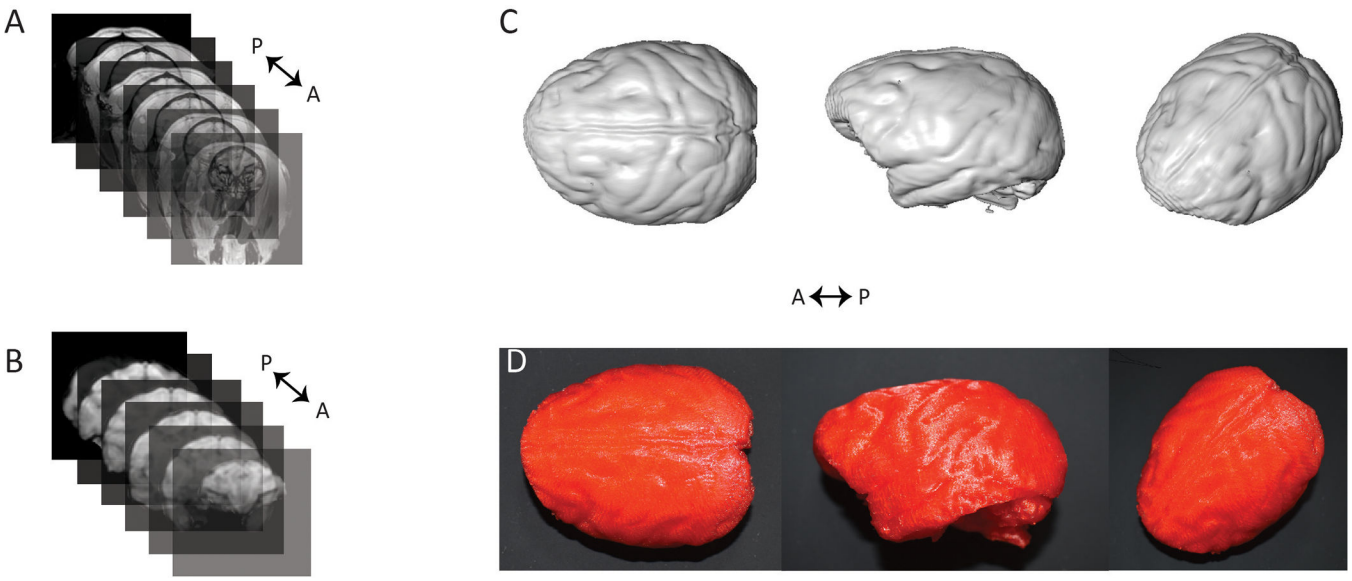

Figure 1: Models of extracted brain.

(**A**) Layered series of T1-QuickMPRAGE coronal slices of the brain of Monkey H. (**B**) Layered series of MR slices of the extracted brain of Monkey H using the BET plugin and Mango software as outlined in the Methods section. (**C**) Axial, sagittal, and skewed view of a model of the gray matter of Monkey H created using the surface building functionality in Mango. (**D**) Axial, sagittal, and skewed view of a 3D printed model of the gray matter of Monkey H created using a Dremel 3D45 extruding printer.

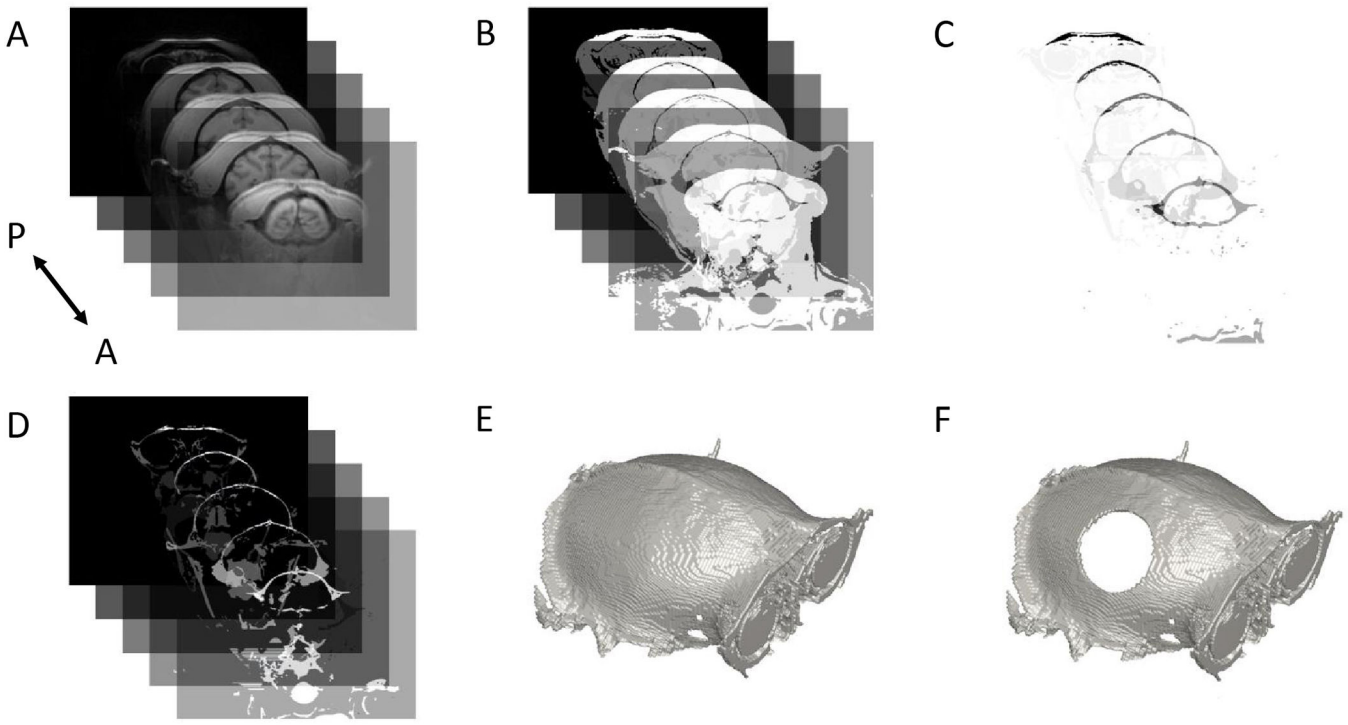

Figure 2: Skull extraction

(**A**) Layered series of T1-QuickMPRAGE coronal slices of the brain of Monkey H. (**B**) Layered series of binary mask after simple thresholding MR slices. (**C**) Layered series of binary mask after removing “musculature layer”. (**D**) Layered series of binary mask of skull after processing as outlined in the Methods section. (**E**) 3D model generated from binary mask. (**F**) 3D model with simulated craniotomy removed.

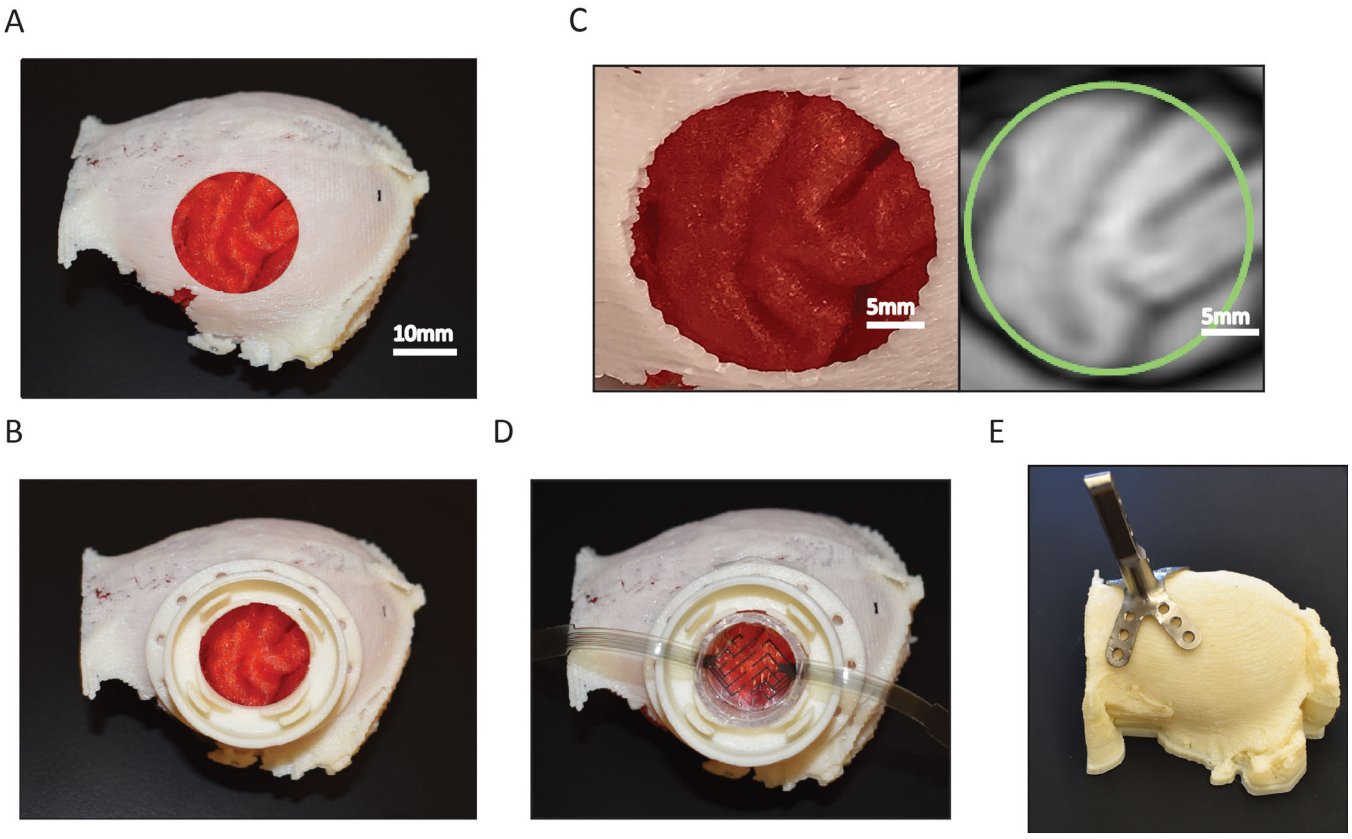

Figure 3: Surgical preparation using 3D printed prototypes.

(**A**) Combination of the 3D printed brain extracted with Mango inside of a 3D printed skull extracted from MRI of Monkey L as outlined in the Methods section. (**B**) Comparison of craniotomy targeting between our 3D models and MR planning in Monkey L. (**C,D**) An example of using our toolbox to prepare for chamber (**C**) and array (**D**) implantation[15]. (**E**) 3D printed model of the skull of Monkey L used for pre-bending the head-post prior to surgery.

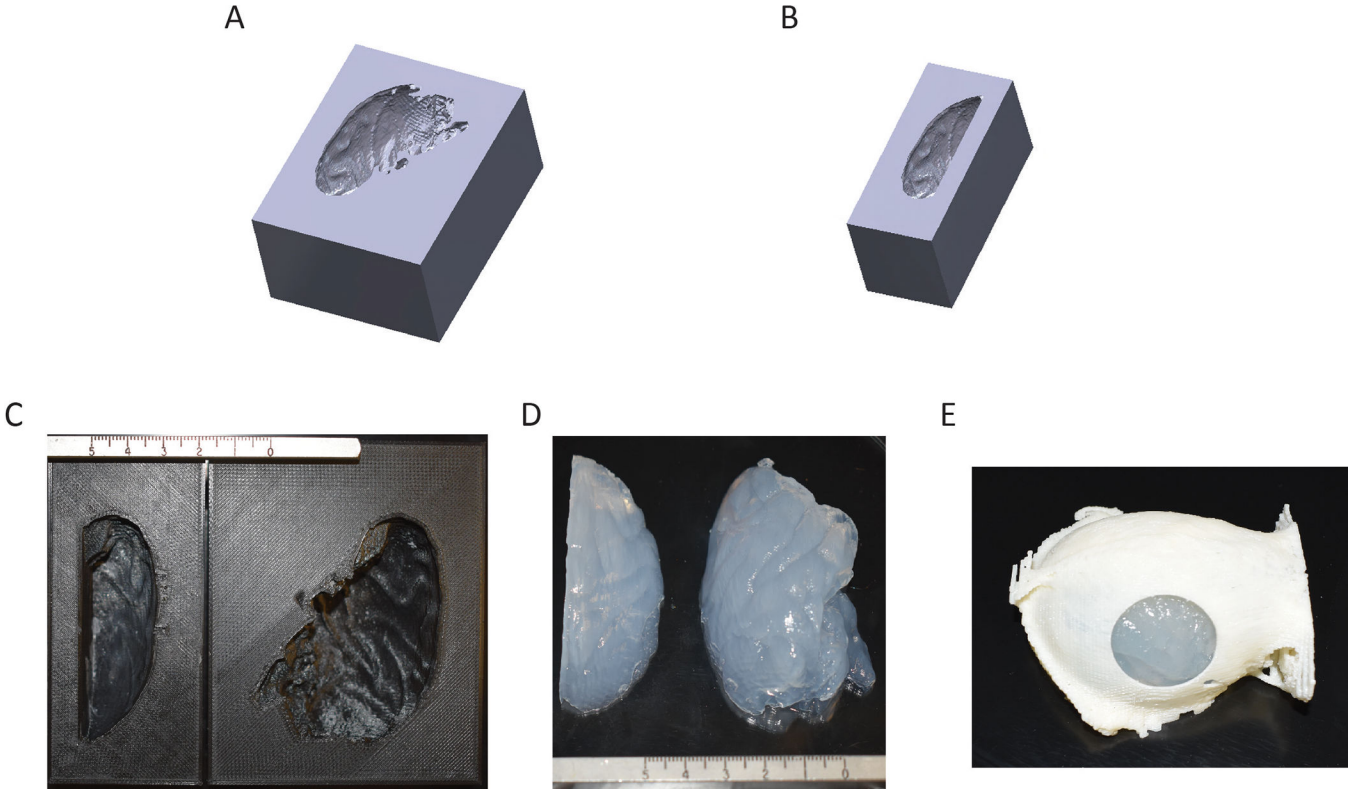

Figure 4: Gel brain modeling.

(**A, B**) 3D model of the mold depicted in SolidWorks for Monkey H. (**C**) 3D printed molds from A and B. Pictured left is a mold used to create the upper portion of the right hemisphere. Pictured right is a mold to create the right hemisphere (**D**) Agarose models of the upper portion of the right hemisphere (left) and the whole right hemisphere (right). (**E**) Agarose model of the right hemisphere placed inside of a 3D print of an extracted skull from Monkey L, demonstrating the accurate representation of the brain and craniotomy.

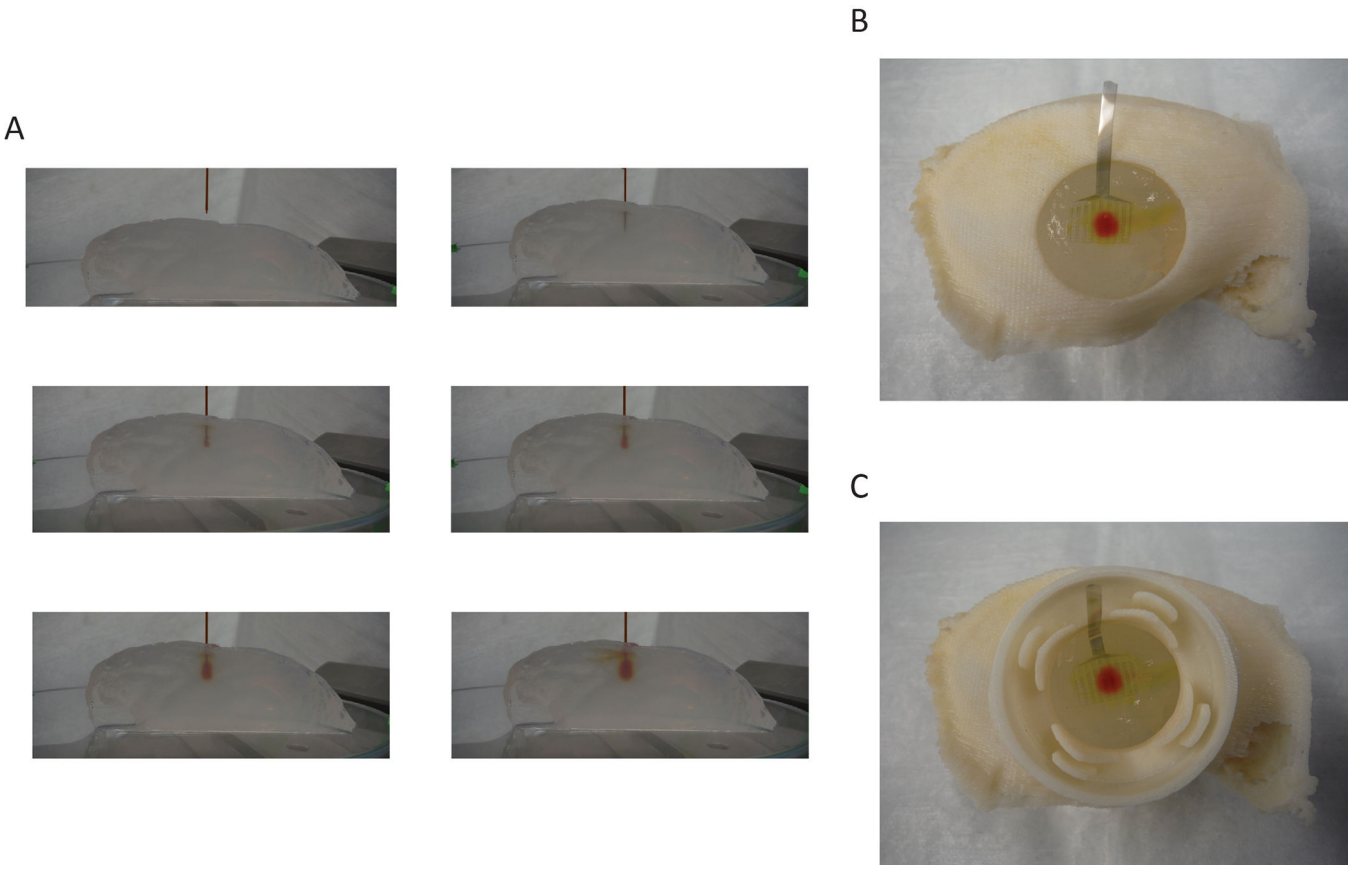

Figure 5: Injection modeling.

(**A**) Time lapse images of the injection procedure. Top left panel pre-insertion. Top right panel post-insertion. Lower four panels show the spread of the dye over time. (**B**) Gel model of a section of the brain positioned within a 3D printed skull with a craniotomy such that injections of food coloring may be observed in relation to the cortical structures and electrode placement. (**C**) 3D print of a chamber fit to the skull and observed in relation to the electrode array, gel model, and injection.

Name of Material/ Equipment	Company	Catalog Number	Comments/Description	
3D Printing Software (GrabCAD Print) (Free)	Stratasys	Version 1.36	Used for High quality 3D printing	
3D Printing Software (Simplify 3D) (Paid)	Simplify3D	Version 4.1	Used for PLA 3D printing	
Agarose	Benchmark Scientific	A1700	Used for making gel brains	
Black Nail Polish	L.A. Colors	CNP637	Used for gel molding	
Cannula (ID 320 um, OD 432 um)	Polymicro Technologies	1068150627	Used to inject dye into gel brain	
Cannula (ID 450 um, OD 666 um)	Polymicro Technologies	1068150625	Used to inject dye into gel brain	
Catheter Connector	B Braun	PCC2000	Perifix for 20–24 Gage epidural catheters; Units per Cs 50	
Dremel 3D Digilab 3D45 printer (standard)	Dremel	F0133D45AA	Used for prototyping in PLA	
ECOWORKS	Stratasys	300-00104	Used to dissolve QSR support structures	
Erlymeyer flask	Pyrex	4980	Used for gel molding	
Ethyl cyanoacrylate	The Original Super Glue Corp.	15187	Used to make combined cannula	
FinePix XP70	Fujifilm	BL04201-101	Used to photograph dye injections	
Graduated cylinder		3023	Used for gel molding	
HATCHBOX PLA 3D Printer Filament	HATCHBOX	3DPLA-1KG1.75-RED/3DPLA-1KG1.75-BLACK	1kg Spool, 1.75mm, Red/Black	
Locust Bean Gum	Modernist Pantry	1018	Gumming agent for gel brain mixtures	
MATLAB (Paid)	MathWorks	R2019b	Used for skull extraction	
McCormick Yellow Food Color	McCormick		Used for dye injection	18006387664
Microwave	Panasonic	NN-SD975S	Used for agarose curing	
MR Imaging Software (3D Slicer) (Free)	3D Slicer	Version 4.10.2	Used for 3D model generation	
MR Imaging Software (Mango) (Free)	Reasearch Imaging Institute	Version 4.1	Used for brain extraction	
MR-compatible stereotaxic frame	Kopf	1430M MRI	Used for dye injection	
Philips Acheiva MRI System	Philips	4522 991 19391	Used to image non-human primates	
Phosphate Buffered Solution	Gibco	70011-044	10X diluted with DI water to 1X	
Pump	WPI	UMP3T-1	Used for dye injection	
Pump driver	WPI	UMP3T-1	Used for dye injection	
Refrigerator	General Electric		Used to preserve agarose gel	
Scientific Spatula	VWR	82027-494	Used to extract gel molds	
SolidWorks (Paid)	Dassault Systemes	2019		
Stratasys ABS-M30 filament	Stratasys	333-60304	Used for high quality 3D printing	
Stratasys F170 3D printer	Stratasys	123-10000	Used for high quality 3D printing	
Stratasys QSR support	Stratasys	333-63500	Used to create supports with ABS model	

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Syn.Flex-S Multicoil	Phillips	45221318125	Used to image non-human primates
Syringe	SGE	SGE250TLL	Used for dye injection