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🌐 In-vivo MRI and CT scanning protocol for non-human primates using a 3D printed MR-compatible stereotaxic frame

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Protocol status: Working
We use this protocol and it's working

ABSTRACT

This protocol describes the methods of using a custom 3D printed stereotaxic frame to acquire MRI and CT scans for nonhuman primates in detailed steps.

High-precision neurosurgical targeting in nonhuman primates (NHPs) often requires presurgical anatomy mapping with noninvasive neuroimaging techniques (MRI, CT, PET), allowing for translation of individual anatomical coordinates to surgical stereotaxic apparatus. Given the varied tissue contrasts that these imaging techniques produce, precise alignment of imaging-based coordinates to surgical apparatus can be cumbersome. MRI-compatible stereotaxis with radiopaque fiducial markers offer a straight-forward and reliable solution, but existing commercial options do not fit in conformal head coils that maximize imaging quality.

We developed a compact MRI-compatible stereotaxis suitable for a variety of NHP species (*Macaca mulatta*, *Macaca fascicularis*, and *Cebus apella*) that allows multimodal alignment through technique-specific fiducial markers.

The computer-aided design files and engineering drawings for the frame used in this protocol are publicly available, with the modular design allowing for low cost and manageable manufacturing.

You can find it here: <https://github.com/isabelazr/Macaque-Stereotax>

This protocol is supplementary to the manuscript:

Liang, L., Rollin, I.Z., Alikaya, A., Ho, J.C., Santini, T., Bostan, A.C., Schwerdt, H.N., Stauffer, W.R., Ibrahim, T.S., Pirondini, E., Schaeffer, D.J., 2024. An open-source MRI compatible frame for multimodal presurgical mapping in macaque and capuchin monkeys. *Journal of Neuroscience Methods* (submitted)

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MATERIALS

Personal Protective Equipment

- Disposable coverall suit with elastic wrist and ankles
- Disposable mask
- Two pairs of examination gloves
- Face shield
- Hair net

Imaging Equipment

- Siemens 7T whole body MRI scanner
- 2nd generation Tic-Tac-Toe radiofrequency (RF) system (Sajewskiet al., 2023; Santini et al., 2021)
- Epica Vimago GT30 animal scanner

Materials

- Non-human primate (Rhesus, Cynomolgus, or Capuchin)
- Sterile saline 0.9% NaCl
- Isoflurane
- Ketamine
- Eye lube
- Lidocaine gel
- 1 ml and 3 ml sterile syringes
- Intravenous (i.v.) extension set
- 22G i.v. catheter
- Tracheal tube
- Absorbant bench pads
- Cotton swabs
- Electric shaver
- Vitals monitoring system
- 3D printed stereotaxic frame (with ear bars and eye bars that fit the animal)
- Contrast solution for stereotax fiducials (see Guidelines for details)
- Plexiglass animal bed
- Bed sheets to keep animal warm
- Vitamin E capsule
- Velcro straps to stabilize animal on CT table

References:

3D printable stereotaxic frame: <https://github.com/isabelazr/Macaque-Stereotax>

Sajewski, A., Santini, T., Tiago, M., Berardinelli, J., Ibrahim, T.S., 2023. Comparison of the Optimization of a 60-channel Transmit Coil in pTx and sTx mode at 7T. Presented at the ISMRM.

Santini, T., Wood, S., Krishnamurthy, N., Martins, T., Aizenstein, H.J., Ibrahim, T.S., 2021. Improved 7 Tesla transmit field homogeneity with reduced electromagnetic power deposition using coupled Tic Tac Toe antennas. Sci Rep 11, 3370. <https://doi.org/10.1038/s41598-020-79807-9>

BEFORE START INSTRUCTIONS

- 1. Confirm IACUC approval and trainings for all personnel involved in the procedures.**
- 2. Check that the fiducial channels in the earbars and eyebars are filled with the appropriate contrast agent.**
 - MRI: CuSO₄ copper sulfate solution
 - PET: ¹⁸F fluorodeoxyglucose
 - CT: iodine solution
- 2. Check the earbars, eyebars, and pallet bars are the appropriate size for your animal.**
- 3. Confirm the availability of required PPE and materials.**

Preparation of the animal

- 1** Transfer the animal to the prep/procedure room close to the imaging room, awake, with cage covered.
- 2** Anesthetize the monkey with ketamine (10 mg/kg) in the home cage. Transfer the animal to the prep table.
- 3** Shave one calve of the animal using an electric shaver/clipper. Palpate the calf muscle and identify the saphenous vein.

- 4 Clean the skin with an alcohol pad. Using a 22-G i.v. catheter, cannulate the vessel and flush with saline.
- 5 Intubate the animal with a 3.5 mm to 5 mm (inside diameter) tracheal tube, according to the size of the animal.
- 6 Maintain the animal on isoflurane anesthesia (e.g. 1.5-2% with an oxygen flow of ~ 0.3 liters/min).
- 7 Lay the animal on the table in a supine position. Place the head of the animal in the stereotaxic frame. Need 2-3 people.
 - 7.1 Prep the tips of the earbars with the lidocaine gel to minimize pain. Apply eye lube to the animal's eyes.
 - 7.2 With the earbar handles attached to the earbar tips, place the earbars inside the ear canals of the animal. Make sure isoflurane is still well connected to the tracheal tube. May need to detach before placing the animal, and reattach from the top of the frame.
 - 7.3 Slide the earbars inside the earbar clamps. Push the two earbars towards the middle while checking the markings on the earbars. When the markings on either earbars are the same, the animal is centered in the frame. Now, tighten the earbar clamps.
 - 7.4 Place and tighten the eyebars and pallet bar. Check that the animal's head is fixed tight in the frame.

Acquiring an MRI scan

- 8 Attach a vitamin E capsule on the left side of the animal's head with tape.
- 9 Line the plexiglass animal bed padded with a cushion and sheets. Since the animal will be in the scanner for over an hour, this will help avoid sores and keep them warm.
- 10 Place the animal in the animal bed, with the head outside the bed, and place the vital monitoring sensors on the animal. Then place the animal bed onto the MR scanner bed. Make sure to remove all magnetic objects before entering the scanner room. The isoflurane and vital monitoring machines should be kept in the observation room.
- 11 Secure the stereotaxic frame to the Tic-tac-toe RF coil with 3D printed brackets and nylon screws. Attach the saline bag to the top of the scanner.
- 12 Slide the scanner bed into position, while making sure the cables and isoflurane tubes are long enough.
- 13 **T1-weighted image:** 400 μ m isotropic, TR/TE: 6000/3.7 ms, FOV: 148×148×96 mm
2DSWI image: 154 μ m in-plane and 1.5 mm thickness, TR/TE: 1650/14 ms, FOV: 128×128×79.5 mm, flip angle of 60 degrees
- 14 Double check the images for good contrast for structure of interest, and visibility of frame fiducials as well as vitamin E capsule.

- 15 Allow animal to recover from anesthesia (~15 min) before placing back in cage for transportation to home facility/cage.

Acquiring a CT scan

- 16 Place the animal directly onto the animal CT scanner table, in supine position. Use velcro straps to hold across the shoulder and hips of the animal to keep them stable. The flat bottom of the stereotaxic head frame should be placed as straight and flat on the table as possible. Use some foam or padding if necessary, to stabilize the frame.
- 17 Move the scanner table to center the laser cross-hair of the scanner on either side of the earbars, and also the midline of the stereotaxic frame.
- 18 Acquire the CT scan at 250 μ m isotropic resolution, and bowtie filter, FOV covering from a few centimeters above top of head down to the shoulders.
- 19 Double check the images for visibility of frame fiducial markers.
- 20 Allow animal to recover from anesthesia (~15 min) before placing back in cage for transportation to home facility/cage.