Jun 09, 2025

🌐 Structural MRI Using the University of Washington 14T Vertical Bore Bruker MRI

DOI

dx.doi.org/10.17504/protocols.io.3byl4j8p2lo5/v1

Yoni Browning[1], Galen Lynch[1], Josh Siegle[1]

[1]Allen Institute for Neural Dynamics

Allen Institute for Neural...

Yoni Browning
Allen Institute for Neural Dynamics

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

Created: March 25, 2023

Last Modified: June 09, 2025

Protocol Integer ID: 79441

Keywords: Magnetic Resonance Imaging, Electrophysiology, Neuroscience

Abstract

This protocol describes the process of obtaining pre-insertion MRI images using the University of Washington 14T vertical bore Bruker MRI. These structural scans allow for precisely targeting acute electrophysiology recordings in head-fixed mice beyond what is possible using skull landmarks alone.

Guidelines

Only perform this procedure in accordance with IACUC and veterinary requirements.

Materials

To Prep Headframe:

GUM Dental Wax

For preparing Manganese Injectable:

⊗ Manganese(II) chloride tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #M3634

⊗ BICINE **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #B8660

⊗ Bicine **Fisher Scientific Catalog** #BP26461

⊗ NaOH **Contributed by users**

⊗ Lactated Ringers Injection, USP, Preservative-Free, Baxter **Henry Schein Animal Health Catalog** #059380
(or similar)

⊗ Apera pH Calibration Buffer Solution Kit **Amazon Catalog** #B01N0QVLLD

Syringe Filters (e.g.):

Equipment	
Nylon Syringe Filters	NAME
0.2 μm	TYPE
Acrodisc, Pall Corporation	BRAND
AP-4436	SKU
https://shop.pall.com/us/en/products/zidAP-4436	LINK

Sterile septum vial (e.g.):

Equipment

Acuflow Systems Sterile Empty Vial (SEV), 10 mL, 20mm clear glass with silver cap | NAME

Sterile Septum Vial | TYPE

ALK | BRAND

SEV1020S-1 | SKU

https://www.fishersci.com/shop/products/10-ml-20-mm-25-pk-silver/NC1741084 | LINK

Digital pH meter (e.g.):

Equipment

PH700 | NAME

Digital Benchtop pH Meter | TYPE

APERA | BRAND

B01N43WRY1 | SKU

https://www.amazon.com/Apera-Instruments-Benchtop-Calibration-Electrode/dp/B01N43WRY1/ref=sr_1_6?crid=1K8BDA5TJAXEZ&dib=eyJ2IjoiMSJ9.vSZ-XvnOy0WBYHw4fNbjF1bmNWpXnGvY9-hsb8fE9aYMD6roWM1cSxFzzzPC9HFLechdCFGPOdmxT6muVjhJvcxrDdHMe9A0UaNimPJCXI4CIQySJD6PfT48XQ | LINK

Microbalance (e.g.):

Equipment	
GH-252 Compact Bench Scale	NAME
microbalance	TYPE
A&D	BRAND
9NVE8	SKU

Powder hood (e.g.):

Equipment	
PowderSafe 700	NAME
Powder Hood	TYPE
PowderSafe	BRAND
21A00M354	SKU

Beaker that can hold ⚗ 100 mL
Sterile container that can hold ⚗ 100 mL
Clean stir bar
Weigh boats
Sterile spatula (e.g.):

Equipment	

Spatula with Narrow Spoon/Spoon, Sterile	NAME
Spatula for weighing	TYPE
Corning	BRAND
3004	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/General-Labware/Spatulas/Disposable-Anti-static-Spatulas/p/3004	LINK

For filling fiducials during scan:

⊗ Systane Nighttime Lubricant Eye Ointment **Amazon Catalog** #B001G0ML14   or other Vaseline substrate.

Equipment	

Syringe 1ML Luer Lock	NAME
Syringe	TYPE
Fisher	BRAND
14-823-30	SKU

Equipment	
25g Blunt 0.5In Sterile Needle	NAME
Needle	TYPE
Fisher	BRAND
NC0481462	SKU

For mouse care during scan (in addition to equipment provided by scanning facility):
Custom MR compatible nose cone (see text)
Custom MR compatible headframe holder (see text)

For Data Transfer:
USB-C flash drive

For Cleanup and Transportation:
Cargo van, e.g.:

Equipment	
Transit Van	NAME
Van	TYPE
Ford	BRAND
NA	SKU
https://www.ford.com/commercial-trucks/transit-cargo-van/	LINK

Mouse transfer cage, e.g.:

Equipment	
Shipping Container	NAME
Mouse transfer cage	TYPE
Jackson Lab	BRAND
NA	SKU
https://www.jax.org/jax-mice-and-services/customer-support/shipping-and-receiving/shipping-containers	LINK

Mouse transfer bin, e.g.:

Equipment	
Clear Storage Box	NAME
Box	TYPE
Sterilite	BRAND
S-14599	SKU

Animal handling hood (e.g.):

Equipment	
Phantom2	NAME
Animal handling hood	TYPE
Allentown	BRAND
NA	SKU

Ratchet straps (e.g.):

Equipment	
Uline Retractable Ratchet Tie-Downs	NAME
Ratchet Strap	TYPE
Uline	BRAND
H-9706	SKU

Pre-empt or other H202 cleaning solution.

⊗ PreEmpt Multi-Surface One Step Disinfectant Cleaner **Amazon**

Safety warnings

⚠️ Manganese (signal word danger) is toxic if swallowed, causes serious eye damage, may cause damage to organs (brain) through prolonged or repeated exposure, is harmful to aquatic life, and is toxic to aquatic life with long-lasting effects. It should be handled with care using local Environmental Health & Safety guidelines. This includes PPE (gloves/lab coat), engineering measures such as measuring the powder in a hood, and proper disposal.

Ethics statement

Research-focused rodent behavior must be conducted according to internationally accepted standards and should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s).

This protocol has been approved by the Allen Institute Animal Care and Use Committee (IACUC).
PHS Assurance: D16-00781
AAALAC: Unit 1854

Before start

Complete the whole hemisphere craniotomy surgery, but substitute a zirconia headframe for the standard titanium version.

Titanium headframes will cause MRI artifacts. This procedure requires a zirconia headframe. Prep the zirconia headframe for surgery by coating the outside of each of the fiducial holes with a thin layer of dental wax. This will prevent Metabond from wicking into the headframe holes during implantation, which would limit our ability to register the MR scan. (See "Prepare for MRI scans" for details about the headframe).

After surgery, recover the mouse according to the surgical and IACUC protocols.

Prepare MnCl$_2$ solution for injection. `1h`

1   Mix `[M] 100 millimolar (mM)` MnCl$_2$ solution. (These steps are from section 3.1.1 of Massaad and Pautler (2011).)   `1h`

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>
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1.1   Create `100 mL` of a `[M] 100 millimolar (mM)` bicine buffer solution by mixing `1.63 g` bicine into `100 mL` MilliQ water in a beaker.

1.2   Place clean stir bar into the bicine solution and place on a stir plate. With a calibrated pH meter, bring the bicine buffer solution to `pH 7.4` using `[M] 1 Molarity (M)` NaOH.

1.3   Sterilize the bicine buffer solution using a `0.2 µm` syringe filter into a sterile container that can hold at least `100 mL` .

1.4   In a hood, aliquot `5 mL` of the sterile bicine into a sterile container. Using sterile tools, measure `98.95 mg` of MnCl$_2$ with a microbalance. Mix the MnCl$_2$ into the bicine aliquot.

1.5   Using a sterile syringe and needle, transfer MnCl$_2$ solution to sterile septum vial using sterile techniques.

2   The injectable should be refrigerated at `4 °C` . Discard when expired according to local IACUC. Label accordingly.

Inject mice with MnCl$_2$ solution, 18-24 hours prior to imaging `2h`

3   Weigh mouse.

4   Calculate the dose of $MnCl_2$ injectable needed for 66 mg/kg of manganese. Multiply the mouse weight by   🧪 3.3348 µL per gram of mouse weight . If the mouse has an implanted headframe, subtract the weight of the headframe from the mouse weight before calculating the dose.

5   Using sterile technique, draw the volume of $MnCl_2$ injectable calculated above into an insulin syringe. Perform an intraperitoneal (IP) injection of the $MnCl_2$ solution.

2m

> **Note**
>
> Do NOT anesthetize the mouse with isoflurane prior to injection. This can interact with $MnCl_2$, and increase symptoms of discomfort.

6   It is normal for mice to be lethargic/uncomfortable after $MnCl_2$ injection. These symptoms typically resolve themselves within a few hours. Work with your IACUC and veterinary staff to identify symptoms of concern and establish protocols to aid in recovery. We recommend the following:

2h

- Provide thermal support to the mouse for a minimum of 30 minutes using an electric heating pad or chemical heat pack.
- Monitor mice for signs of unusual pain/discomfort, and to ensure continuous improvement.
- For mice not on water restriction, provide fluid support to the mouse via subcutaneous injection of   🧪 0.25-.5 mL   of sterile LRS (Lactated Ringers Solution) or saline.

Prepare for MRI scans

1h

7   Place each mouse in a disposable transport cage. Transport the mice to the imaging facility. For transport, transfer cages should be placed in a large plastic bin that is secured with ratchet straps in the back of an approved cargo van.

8   Confirm the mouse ID number by matching ear tags/tattoos to the ID number on the cage.

9   Anesthetize the mouse with 2-5% isoflurane using an induction chamber.

10   Apply eye lubricant to the mouse's eyes.

11   Secure the MRI-compatible nose cone. While some scanners have prefabricated cones, the headframe on our mice interferes with the commercially available options from Bruker. We find that a rubber pipe bulb, cut to accommodate the mouse's nose and teeth, works well. This can be secured to the mouse's face using strips cut from thin surgical tubing. The image below illustrates this setup and how it is attached to the mouse as well as to the anesthesia system.
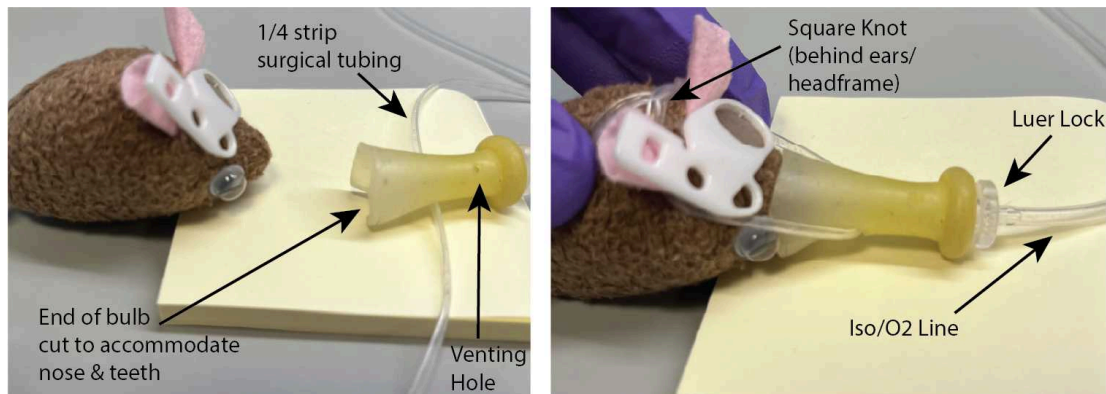

     Mouse "nose cone" and the process of attaching it.

12   Fill the fiducial holes in the mouse's headframe with degassed white petroleum-based eye lubricant (e.g. Systane Nighttime lubricant) or Vaseline using a 25 gauge blunt-tipped syringe. To do this, you will likely need to remove any protective wax placed over the holes prior to surgery; this can be done using any sharp-tipped needle. Backfill the holes, taking care to avoid bubbles in the holes as these will limit the ability to see the holes in the final scan. Good technique for doing this is to perturb the Vaseline mixture as you fill by moving the syringe in the hole so that any bubbles that do form are forced out. Additionally, spread eye lubricant or Vaseline onto the lower plane of the shank of the head bar.

During post-hoc analysis, these fiducial fills will be used to align the scan to the headframe holder on the rig. The lower plane of the headframe and other geometry can be used to augment incomplete hole fills. See Registering MRI Volumes to an In Vivo Electrophysiology Rig for more information. If you are having trouble localizing the

headframe in your scans, it can be useful to spread eye lubricant around other salient geometric features on the headframe so that you have more data for alignment.
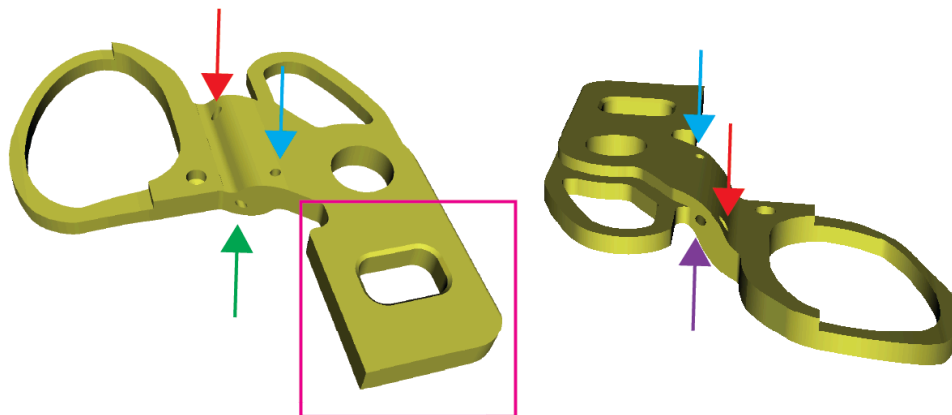
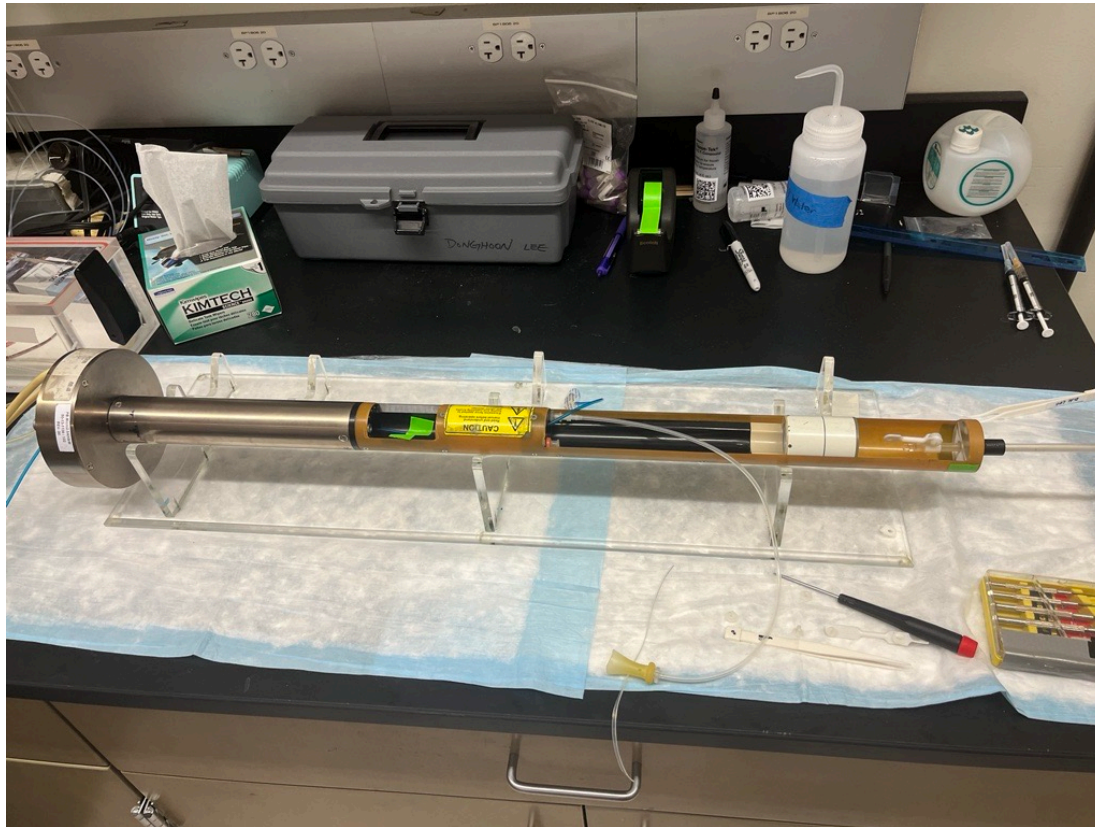Headframe model.
Red = Anterior Vertical hole
Blue = Posterior Vertical
Green = Posterior Horizontal
Purple = Anterior Horizontal
Pink = Shank, lower section should be coated.

13   Load the mouse into the RF coil, securing the headframe to a holder if using one.

RF Coil (no mouse).

13.1   Our headframe holder for the Bruker 14T Vertical bore magnet is illustrated below, though modifications may be needed to affix other headframes or other scanners. In our case, we use a plastic nut (A) and screw (B) to affix a plastic holding bar (C) to a stabilization bolt hole on the mouse's headframe (D). The plastic bar is positioned roughly in line with the protruding handle on the mouse's headframe. The screw should be tight enough that the mouse does not move, but care should be taken not apply to much force as the plastic interface can strip easily. The exact position here is not strictly important - fine alignment will use the fiducial holes filled above; this setup is just to prevent the mouse from moving during the scan.
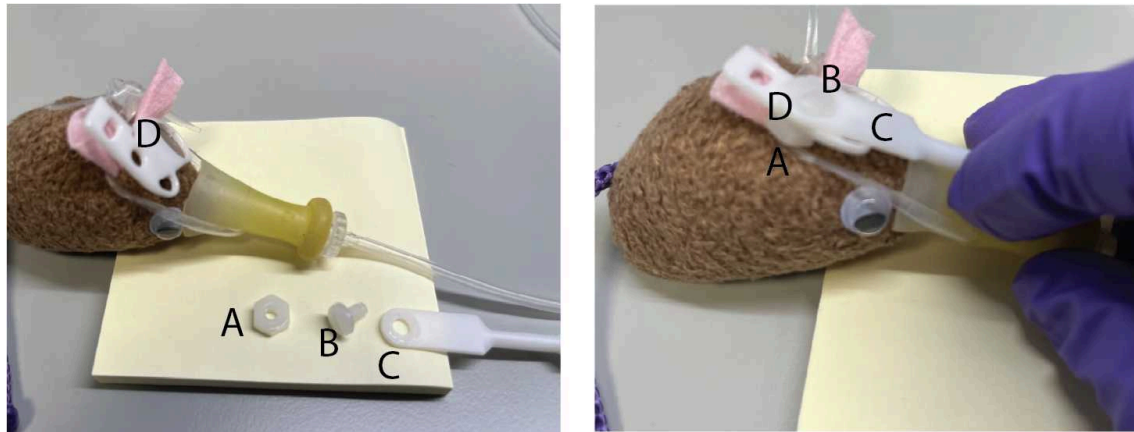
Schematic showing stabilization setup.

13.2   To position the animal in the center of the coil, it can be helpful to measure the distance from the center of the animal's brain to a bolt on the headframe holder. Because these bolts will stick out of the coil once the animal is loaded, they can be used to approximately center the mouse's brain in the coil.
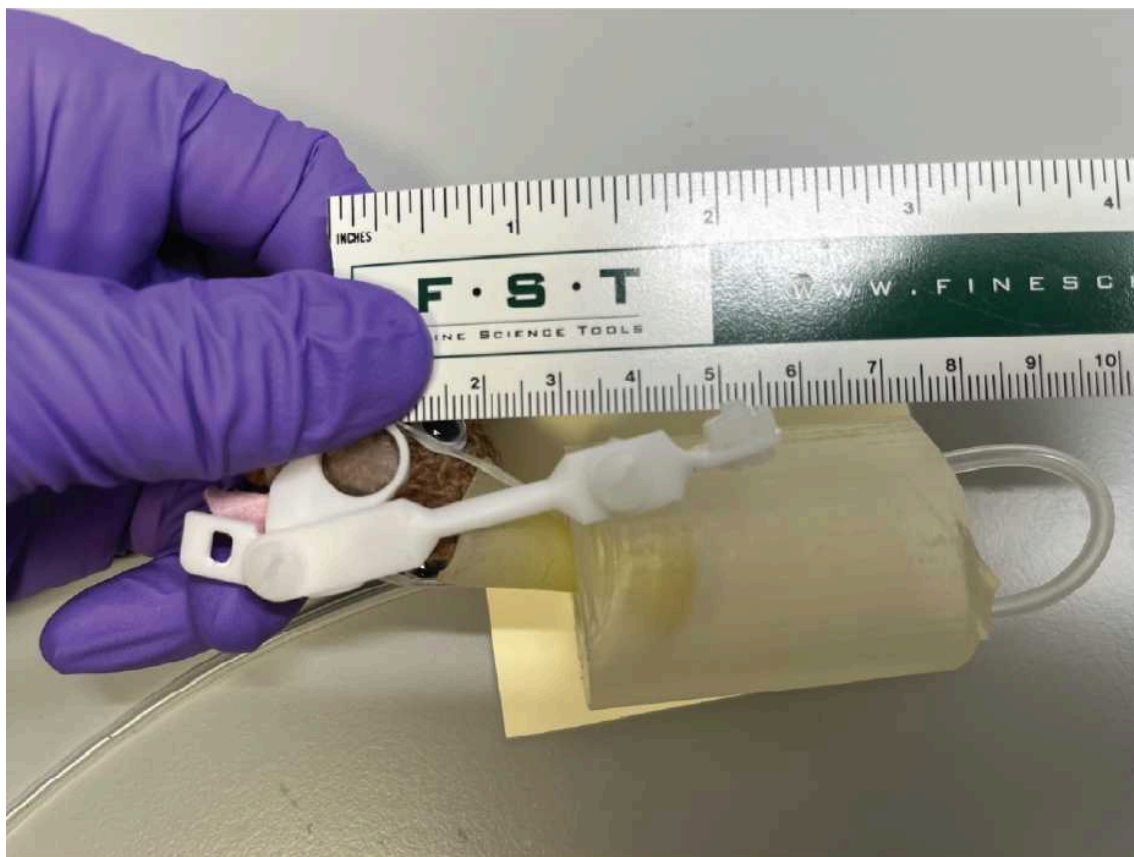
Though not shown in this illustrative example, the RF coil has its center labeled. (see below)

13.3   Because the headframe is wider than the mouse's head, the animal needs to be loaded into the RF coil at an angle. The mouse should be tilted so that the holder is pointing straight, and the animal's head is tilted to the right. The gas line for the anesthesia should be passed through the coil ahead of the mouse and should sit under the animal when it is loaded into the scanner (if it is placed to the side, it can cause "fold over" or "wrap-around" imaging artifacts in the scan. Take care when loading and unloading the animal into the coil - rough handling here can put unnecessary strain on the animal's neck.
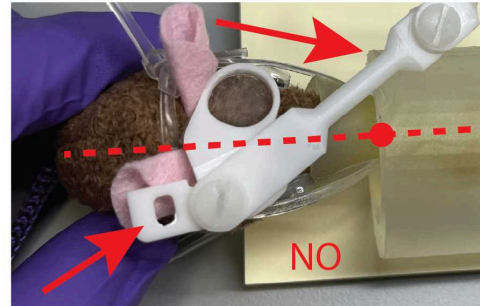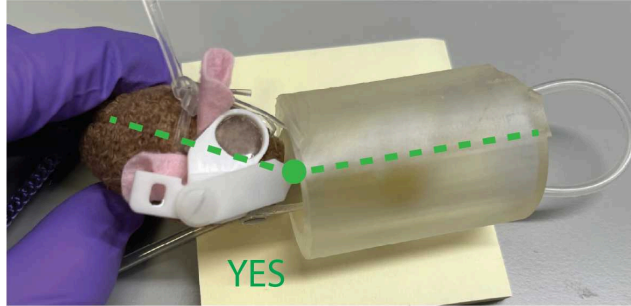
Correct loading angle into the RF coil.

13.4   Once the animal is fully loaded into the coil, a trained MRI technician can finish securing the animal and place a breathing pillow to monitor respiration during the scan.
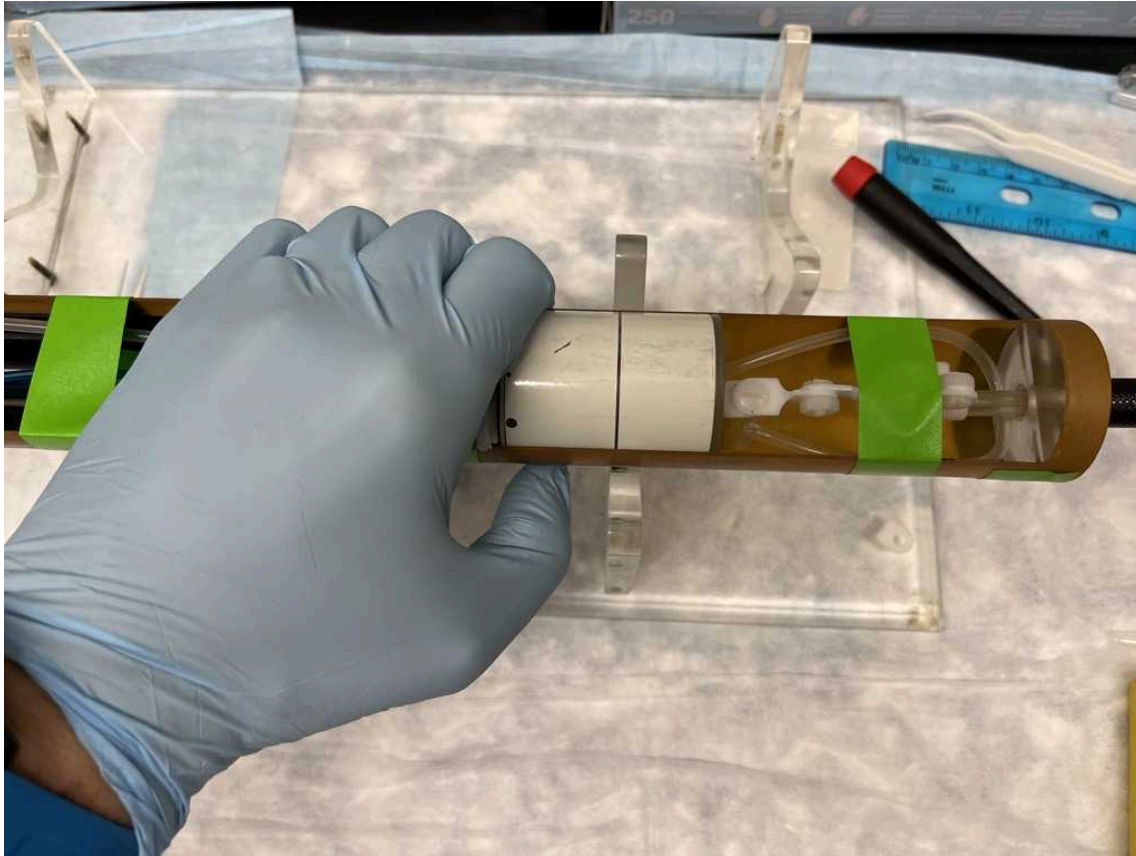
RF coil with mouse securely held in place.

Perform the primary MRI scan

`2h`

14   The trained MRI technician will operate the scanner. Work with them to achieve the following. Note that the order of these steps may change on different hardware/software, that some steps may be automated, or that additional steps may be required depending on the equipment used. If any steps here are unclear, consult the manual for your equipment.

- Adjust the position of the mouse in the scanner to achieve the best scan quality. Ideally, the brain will be as centered in the scan as possible while still allowing the headframe holes to be visualized.
- Perform matching and tuning (consult your MR technician and/or your scanner's manual for details on this step).
- Determine the scan volume desired, being sure to include both the brain and the positioning holes in the headframe. To do this, we typically collect axial and coronal scans with large slice thickness and use these to confirm that all needed features are included.

- The readout direction for the high-resolution 3D scan should be chosen to align with the mouse's AP axis to avoid signal aliasing. In the non-readout axes, place saturation bands around the scan volume as needed to limit aliasing. This will allow for a smaller scan volume while minimizing aliasing.
- We recommend taking a low-resolution scan prior to the "full" 3D scan list in the next step. This will allow you to confirm that the headframe holes have adequate fill and that the scan volume is correct.
- If the hole fills are incomplete or show artifacts, take the mouse out of the scanner and repeat ⮌ 12-14 , being sure to avoid air bubbles in the Vaseline.

15   Perform the full 3d scan. For the UW MRI scanner, typical parameters are as follows. Work with your scanner operators to validate that these parameters are correct for your equipment and needs. Your MRI Technician will set these parameters and can load parameters from previous scans.

1h 30m

Parameters:
1. Scan Type: RARE (fast spin echo)
2. Resolution: 100 µm isotropic
3. Number of Averages: 2
4. RARE Factor: 4
5. Echo Time: 5.333 ms
6. Effective TE (time to echo): 10.666 ms
7. Repetition Time: 500 ms

> **Note**
>
> The precise parameters of these scans are subject to change. Parameters used will be recorded in the Bruker image files.

16   Remove mouse from scanner (done by MRI operator) and recover mouse with thermal support. Return the mouse to the transport cage once the mouse begins to ambulate.

17   Repeat scanning procedure for all mice in a group, sanitizing any surfaces the mouse contacts after each scan.

18   Transfer the data to a USB drive.

Transport mice back to home vivarium

1h

19 Transport mice back to home institute and follow IACUC protocols for returning mice.

20 Transfer data to appropriate storage location.

Protocol references

Massaad, C. A. & Pautler, R. G. Manganese-Enhanced Magnetic Resonance Imaging (MEMRI). *Methods Mol Biol* **711**, 145–174 (2011).