

2.11. Magnetic resonance imaging (MRI) in rodents

Authors: Alison Blain, Elizabeth Greally, Corrine Betts, Anna Bratasz

A. OBJECTIVE

Assessment of right and left ventricular function in mice using MRI*.

*This SOP concentrates on mouse cardiac MRI. There will be some small changes to acquisition parameters for rat imaging. In addition, a larger rat gradient and rat volume coil (often 72mm) is used for rat cardiac imaging.

B. CAUTIONS

- The heating system should be turned on in advance to ensure that there is no unnecessary cooling of the first mouse scanned.
- Ideally, the magnet should be 'tuned and matched' after positioning of each mouse.
- Quality of the final image depends on SNR (coil sensitivity) and magnetic field homogeneity. To reduce acquisition time the smallest possible coil should be used (or any coil with higher sensitivity). A consequence of using the high magnetic field is presence of susceptibility artifacts. For higher magnetic field the smallest possible shimming coils should be used to obtain the best homogeneity of B₀ and reduce susceptibility artifacts.
- Care should be taken to obtain a good shim to establish magnetic field homogeneity as this has significant impact on image quality.
- **Scanning parameters given in this SOP are example parameters based on a 9.4T Bruker system. Scanning parameters should be optimized for the specific system used.**

C. MATERIALS AND EQUIPMENT

- A number of systems are available and can be used to generate comparable data, some examples include:
 - 11.7T MR vertical bore system (Magnex Scientific, Oxon, UK)
 - 7T horizontal bore Varian microimaging system equipped with a 12-cm microimaging gradient insert (maximum gradient 40 gauss/cm), (Varian Inc., Palo Alto, CA, USA)) and warm air blower. 40mm birdcage coil (Rapid Biomedical, Wurzburg, Germany) or 39 mm diameter quadrature birdcage volume coil (Rapid Biomedical GmbH). *A smaller coil (32 mm diameter) may be used for mice up to approximately 25 g.*
 - 9.4T BioSpin Bruker Co. system (9.4T) horizontal bore system with mouse gradient and mouse volume (35mm, 1H) coil available with mouse holder and tubing with circulated warm water which can be placed over the animal's body to maintain body temperature in the physiological range.

- 500 MHz Ultra Shield Bruker Co. vertical bore system (11.4T) with mini-imaging modality equipped with 30 mm mouse volume coil (1H) with warm air blower.
 - 7T Bruker BioSpin system
- Mouse/rat cardiac function can be performed with a gating system such as SA instruments, Inc., Stony Brook, NY (acquisition is gated to animal ECG signal) or using the IntraGate (self-gating) future (“gating” is performed after the acquisition).
 - For monitoring of ECG, temperature and respiratory rate a sled may be used (Dazai Research Instruments, Toronto, Canada) or separate electrode probes, respiratory cushion and rectal or surface temperature probe.
 - Gel pad for ECG contacts if sled is used.
 - Veet depilatory cream.
 - 70% ethanol.

D. METHODS

1. The mouse is anaesthetized in 5% isoflurane, 0.5-2L/min oxygen in an anaesthetic chamber.
2. The mouse is shaved and dehaired in the region of the ECG contacts (if using a sled for monitoring) then transferred to the scanner. To maintain anesthesia, set the nosepiece at 1.5-2% isoflurane, 0.5L/min oxygen.
3. The mouse is placed in the prone position on the custom-built sled. Alternatively, electrode probes are attached (metal pads coated with gold) to left forepaw and right hind paw of the mouse. The probes are attached using an electrode gel/paste and secured with micro pore tape. Alternatively one can use ECG needles by inserting them into the left forepaw and right hind paw. A temperature probe is inserted into the rectum of the mouse.
4. The mouse is slid inside the coil. ECG should be observed during this time to ensure that it is stable.
5. Images can be acquired using a number of different consoles and software packages that come with the scanner (such as the Bruker console running Paravision 2.1.1 (Bruker Medical, Ettlingen, Germany) or a Linux system running VNMRJ).
6. Correct placement of the mouse is confirmed by means of a three plane Scout scan.
 - *Example parameters: 3-orthogonals, TR=125 ms, TE=3.1 ms, FA=30°, n=1, FOV = 40 mm*40 mm, slice thickness = 1.0 mm, matrix size = 128 x 128), Gating - ON*
7. A wobble (tuning and matching of the coil) and shim are acquired (during shimming magnetic field homogeneity is established. Good homogeneity of magnetic field B₀ is

important for good quality images (high SNR) and reduction of susceptibility artifacts which are common and magnified with higher magnetic field).

The left and right ventricles are imaged by taking a contiguous stack of short axis cine images in 1mm increments. It is necessary to perform several steps in order to achieve the true short axis.

8. Specifications for cine: Example parameters: TR=16 ms, TE=2.8 ms, FA=20°, aver=2-4, coronal, (Movie cycle- ON, motion suppression- ON), FOV=3*3cm, matrix 256*256 (or 256*192)
 - a. On the scout scan, position four 1mm slices with a 0.5mm gap, axially across the thorax to cover the ventricles of the heart. Scan.
 - b. Using the '4 slice axial scans' from (a), orientate a single slice so that it bisects the left and right ventricles as consistently as possible in all 4 slices. Scan. This should show an approximate 4-chamber long axis image.
 - c. Using the scan from (a) position a single slice to pass through the left ventricle only i.e. at right angles to the scan in b. then on the scan obtained in b position a single slice so that it passes through the aorta and apex of the left ventricle Scan. This should give an approximate 2-chamber long axis view.

Ensure the long axis scan is orientated correctly by looking at the slice calculation on the short-axis scan (obtained from 8.b). If the slice does not bisect the left ventricle, undo the scan, position the slice correctly and rescan.

9. Contiguous cine images at 1mm increments.
Specifications for cine: *Example parameters: TR=8 ms, TE=2.8 ms, FA=18°, aver=4-8, axial, Movie cycle- ON, motion suppression ON, FOV=3*3cm, matrix 256*256 (or 256*192).* Set heart rate according to ECG (10 BPM higher than the actual heart rate). Using the long-axis scans, draw an axis line that passes through the aorta and apex of the left ventricle, and orientate the slice at right angles to this axis. 12x 1 mm slices should be sufficient to cover the whole of the right and left ventricles.
10. Recover mouse. Remove mouse from cradle and ensure it recovers fully before returning to its cage.

E. EVALUATION AND INTERPRETATION OF RESULTS

- Image analysis can be achieved using ImageJ software (NIH Image, Bethesda, MD) or Segment software
- **Image J method:** The epicardial and endocardial borders from each scan are drawn using the ImageJ free-hand tool. These measurements are taken at end-diastole and end systole to calculate the left ventricle (LV) mass, LV and right ventricle (RV) end-diastolic volume (EDV) and end-systolic lumen volume (ESV). These measurements allow calculation of stroke volume (SV), ejection fraction (EF) and cardiac output (CO).
- **Equation 1:** $SV = EDV - ESV$
- **Equation 2:** $EF = \frac{SV}{EDV} \times 100\%$

- **Equation 3** (HR stands for heart rate): $CO = SV \times HR$
- **Segment Method:** Alternatively FDF files can be converted to matfiles using Matlab and analyzed in freely available Segment software (<http://segment.heiberg.se>).

F. REFERENCES

Elizabeth Greally, Newcastle University

Corrine Betts, University of Oxford, Professor Matthew Wood laboratory.

Anna Bratasz, Small Animal Imaging Core, The Ohio State University.