Objective	To provide a reference for researchers considering the use of MRI as an imaging tool in Animal Use Protocols
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Introduction

The MRI, supported by the Gerald Choa Neuroscience Institute (GCNI), is utilized to provide advanced imaging support for various scientific research applications. It plays a crucial role in non-invasive imaging of mouse models, aiding in the study of anatomical and functional changes over time. This technology is essential for monitoring disease progression, evaluating treatment efficacy, and conducting longitudinal studies without the need for euthanasia. The MRI's high-resolution imaging capabilities facilitate detailed analysis of internal structures, contributing significantly to the advancement of biomedical research.

The document outlines the ethical considerations, technical procedures, and regulatory compliance requirements for researchers considering the use of MRI services provided by GCNI.

Ethical Considerations

When planning experiments, the application of MRI should be considered for the 3Rs (Replacement, Reduction, and Refinement) principles.

Replacement

- Consideration of Alternatives: Prioritize the use of alternative methods or models that do not involve animal experimentation.
- In vitro studies consideration: Consider utilizing cell culture models for preliminary studies prior to transitioning to in vivo MRI experiments if possible.
- Existing biomedical data: Explore existing biomedical database and analysis techniques to gather relevant information without the need for performing additional animal experiments

Reduction

- Minimal Sample Size: Optimize experimental design to minimize the number of animals required for each study while ensuring statistical significance. Perform power analysis to determine the optimal number of animals if a pilot study has been done.
- Longitudinal Study: Leverage the non-invasive nature of MRI, which allows for longitudinal data collection on individual animals, thereby reducing the overall number of animals needed.

Refinement

- Protocol refinement: Refine MRI protocol intermittently to minimize stress and discomfort experienced by the animals by reducing the number and duration of scans if possible.
- Anesthesia Monitoring: Ensure the depth of anesthesia is adequately controlled, to improve the quality of scans and reduce the need of rescan in case of failure.
- Post-Imaging Care: Provide appropriate post-imaging care and monitoring to minimize any adverse effects resulting from scans.

Regulatory Compliance

The personnel must be appropriately licensed to conduct the MRI procedures under the Animals (Control of Experiments) Ordinance issued by Department of Health.

Safety Considerations

Researchers considering the use of MRI services should reference the Standard Operation Procedures SOPs in their AEEC AUP applications.

A. Safety guidelines herein are adopted from SOP100.01: SMALL ANIMAL MRI/PET PROTOCOL GENERAL GUIDELINE.

1. All procedures on animals should be performed by properly trained personnel. The following practices should be adopted:

    - Avoid eating, drinking, smoking, handling contact lenses, applying cosmetics, or taking or applying medicine.
    - Avoid using sharps whenever possible. Never remove or recap disposable syringes. Use safe needles whenever possible.
    - Keep doors to animal holding room closed.
    - Use precautions to avoid generation of splashes or aerosols.
    - Restrict operations that generate hazardous aerosols to the safety cabinet.
    - Remove gloves and wash your hands after handling animals or animal tissues and before leaving the laboratory.
    - Report all incidents and equipment malfunctions to laboratory attendant.

B. Refer to the University Safety Office General and Chemical Safety Manual: GCS Section 3 for Guidelines.

2. Wear Appropriate Personal Protective Equipment (PPE)

    - Wear all required PPE identified in the Health, Safety and Environmental Assessment approval obtained from the University Safety Office, CUHK (USEO).
        - Wear a laboratory coat.

- Disposable gowns, coveralls, gloves, shoe covers, and head covers should be worn and disposed of prior to exiting experimental area.
- Eye protection should be worn when needed.

3. A strong magnetic field of the MRI presents a potential risk for personnel working in proximity to the scanner

- Small and large ferromagnetic items should not be introduced into the scanner room when the system is magnetized. The items may become projectiles and pose a serious threat to the health and life of those in the room.
- All metal items that are brought into the scanner room for research studies must be checked with a ferromagnetic metal detector.
- Any behavioral, physiological or other devices that are not MR compatible may be damaged
- Strong magnetic field poses hazard to pacemaker wearers and others with medical implants

MRI procedure to be described on Animal Use Protocol Application

Preparation of Animal

1. Transport cages from the animal holding room to the scanner room.
2. Anaesthesia options:
    a. Anesthetize animals in the induction chamber with 3% isoflurane at 1litre/minute flow rate with oxygen as the carrier gas.
    b. Anesthetize mice with Ketamine (100mg/kg) and Xylazine (10mg/kg), i.p. (27 gauge); or rats with Ketamine (80mg/kg) and Xylazine (10mg/kg), i.p. (25 gauge)
3. Tracer or contrast agent injection:
    a. Contrast agents can be administered intravenously in the shielded hood while the animal is under anaesthesia. The recommended maximum volume is 0.2ml for mice, and 0.5ml for rats respectively.
4. Perform the toe pinch test to assure animals are anesthetized, then transport the animal to imaging platform
5. Ophthalmic ointment should be applied to the eyes of the animal to prevent dehydration during anaesthesia
6. Isoflurane vaporizer is set to 1-2% with oxygen as the carrier gas with flow rate of 1 litre/minute to maintain anaesthesia
7. The animal should be preferably placed in supine position with respiratory pad engaged. Imaging platform is placed within the centre of the coil array for imaging.

MRI procedure

- Image acquisition can be initiated once the animal is situated in the coil array.
- Time of scan should be recorded and each imaging session should be limited to 30 minutes.
- The following should be noted during the scan:
  - Animal body temperature is maintained at 37ºC ± 1ºC by supplying warm air around the animal holder with a temperature sensor (as feed-back) located within the animal platform.
  - The animals' respiration is monitored by a pulmonary pad / physiology monitoring system and the isoflurane concentration supplied by the vaporizer is modified to maintain a constant respiration rate (40-50 bpm).

Post-imaging Procedure

1. At conclusion of an imaging session (For recovery)
   - Move image bed to provide access to the animal and coils
   - Transport the animal to their respective cage and place on recovery station with warming pad maintained at 37ºC.
   - Monitor the breathing of animal until they completely recover from anesthesia.
   - Transport the cages back to the rack located in animal holding room.
2. Euthanasia
   - Method of euthanasia must follow the approved protocol submitted to Department of health and AEEC. The general approved dosage is >200 mg/kg sodium pentobarbital solution (i.p.).
   - Death must be verified by assurance of:
     a. Cessation of respiratory and cardiovascular movement by observation at room air for 10 mins or;
     b. By employing a secondary method of euthanasia such as cervical dislocation or decapitation.

Transportation

1. The transportation arrangement for animals entering and leaving the MRI room should be discussed with and approved by LASEC Director on case by case basis.

Animal Holding in Animal Holding Room (G07B)

1. Investigative personnel must identify each cage they intend to care for using the cage labels provided.
2. Food and water will be provided ad libitum by LASEC staff unless prior approval for restriction has been obtained from AEEC.
3. Routine husbandry, including cage change, will be performed by LASEC husbandry staff.
4. Cage identification card must be filled out with up-to-date information, including the date, cage ID, and the name of the PI and contact number of the user responsible for the cage.

5. Light cycle: A 12-hour light/12-hour dark cycle is maintained. Researchers are advised not to enter the mouse room during the dark cycle. If entry during this time is necessary, red light should be used.
6. Temperature and humidity levels are regulated within the range of 20-24°C and 40-60% humidity, if required. These parameters are visibly displayed on the control panel located near the entrance.

Waste Disposal and Decontamination

1. Disposal of biohazardous wastes, sharp wastes and PPE must be disposed of in designated waste containers in the facility.
2. Surfaces of equipment that has been in contact with animal must be decontaminated with 75% isopropyl alcohol.