UBC ACC Guideline on Imaging of Rodents

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Introduction

Imaging studies on rodents can impact the welfare and health of these animals in a number of ways depending on the use of anesthesia and/or restraint, the duration and frequency of imaging sessions, the use of contrast agents or tracers, and other procedures related to animal preparation. Repeated imaging under anesthesia of the same animal can present challenges for animal care ("Refinement"), however, repeated imaging can also reduce numbers of animals, thus promoting "Reduction". Due to the wide range of modalities, disease models, and experimental aims, these guidelines are intended to outline general factors that should be considered when designing animal use protocols incorporating imaging techniques.

Good references for more detailed guidance pertaining to specific anesthetic protocols and imaging techniques include (Gargiulo S *et al*. 2012 a, b; Tremoleda *et al*. 2011; Tremoleda *et al*. 2012).

Key Points

1. **Imaging procedures should not seriously impair the health of the rodent's undergoing the imaging.**
2. **The health of an animal undergoing imaging must be assessed using clinical health measures.**
3. **Appropriate supportive care must be provided to maintain the health of the animal, especially when animals are recovered after imaging and undergoing repeated imaging sessions.**
4. **Frequency, duration of imaging must be minimized as much as possible while supporting the scientific goals of the study.**

General Guidelines

Type of imaging technique, frequency and duration of imaging session and choice of species will vary according to the study. For all animals undergoing imaging procedures, these factors must be justified within the ACC protocol. Overall, to achieve humane technique, researchers should **demonstrate, within their animal care protocol, that the requested imaging procedures comply with the ethical and humane use of animals.**

The tolerance of an animal to imaging must be assessed using clinical health measures. Tolerance will include the ability of the animal to maintain vital signs during imaging as well as the ability of the animal to recover from imaging sessions, especially if there are repeated sessions. In cases where animals are tethered, then habituation to the procedure prior to collecting data will be important.

Monitoring of Vital Signs During Imaging Sessions

- Anesthetized or un-anesthetized, restrained animals must not be left unattended.

- Animals should be monitored periodically throughout imaging sessions. Vital signs include heart rate, respiratory rate and effort, body temperature, colour of extremities and oxygen saturation. Imaging sessions should be limited to the duration and frequency where appropriate anesthetic depth can be maintained while maintaining vital signs. The extent of monitoring should be clearly outlined in the protocol.
- Researchers that study rodents as models of disease or that have major pathologies "must take into account the increased risk that is associated with anesthetic agents and techniques." (Gargiulo et al. 2012).
- Reference values in Table 1 can be used as guidelines for normal adult rodents undergoing anesthesia. If one is monitoring these variables, then if recorded values are outside the range, then appropriate actions should be taken to remedy the situation. This may require discontinuing imaging. The following are examples for evaluating changes in vital signs:
    - If heart rate increases by about 20% from baseline (at start of anesthesia), then the animal is likely getting light.
    - If heart rate decreases by more than half of resting rate, then the animal is likely getting too deep.
    - For other mammals, hypotension (poor oxygenation of tissue) occurs when Systolic < 80 mmHg , Mean Arterial Pressure < 60-70 mmHg, and Diastolic < 40 mmHg (Duke and Henke, 2008) and hypertension occurs when Systolic > 180-200 mmHg (Duke and Henke, 2008).

Table 1. Reference Values for Adult Mice and Rats[1]

	Rats	**Mice**
Core Body Temp (^{o}C)	37.5 – 38.5	36.5 – 38.0
Normal Heart Rate (bpm)	300 – 500	300 – 840
Heart Rate under Anesthesia[2]	250 - 400	400 – 600
Normal Blood Pressure mmHg		
Systolic	116	133-160
Diastolic	90	102-110
Mean Arterial (MAP)	93 (86-108)	103
MAP under isoflurane		79
Normal Respiration Rate	75 - 129	90 - 250
Respiration under Anesthesia	60 – 90	80 - 120
Tidal volume	0.26 (0.23-0.30) (ml / 100g)	0.18 (0.09-0.38) ml
Minute volume	27.0 (24.6-28.4) (ml / 100g)	1.46 ml/g/min or 24 (11-36) ml/minute
Pulse Oximetry (room air)	>95%	>95%
Pulse Oximetry (100% O_2)	>98%	>98%
Water consumption	8-11 ml/100 g body weight per day	4-7 ml, or 15 ml /100 g of body weight per day

Food consumption	5-6 g/100 g body weight per day	15 g/100 g body weight per day

[1] Reference values based on the following references. Note reference values may vary with strain, sex and age of animal.

Baumans (2010); Fairchild (1972); Hoyt et al. (2007); Jacoby et al (2002); Kohn and Clifford (2002); Koolhaas (2010); Strohl et al. (1997).

[2] Heart rates under anesthesia can be lower than above values if xylazine or dexmedetomidine are used.

Supportive Care and Recovery from Imaging Session

Appropriate supportive care must be provided to maintain stable health of the animal, especially when animals are recovered after imaging and undergo repeated imaging sessions.

Refer to Surgery Policy on details of recovering animals from anesthesia.

- Animals should be kept warm during and on completion of an imaging session, until full recovery from anesthesia.
- The frequency and intensity of post-imaging health monitoring should increase as animals undergo longer sessions of imaging and more frequent sessions of imaging.
- Supportive care will depend on length and frequency of imaging sessions.
- Regular monitoring of hydration will be necessary for animals undergoing anesthesia and imaging. Hydration can be assessed visually (skin tent present, sunken eyes) but loss of weight is also a good indicator of dehydration in rodents. Therefore, rodents must be weighed on each scanning day and in the days following imaging.
- Animals should be administered subcutaneous fluids before imaging for imaging sessions lasting over 15 minutes and requiring anesthesia. For repeated imaging in one day subcutaneous fluids should be given at the initial session. With repeated imaging animals may eat and drink less in between sessions so supplemental fluids may be necessary in the periods between imaging sessions. As a reference point, 10 ml/kg is recommended for animals undergoing surgeries of duration greater than 15 minutes (UBC Rodent Anesthesia SOP).
- Heat must be provided to all animals undergoing anesthesia. Most anesthetic agents depress thermoregulation. Due to their large surface area and high metabolism rodents are at risk of hypothermia which can be a considerable source of mortality
- If animals are undergoing repeated imaging within one day or on consecutive days, they may need supportive care in between sessions (e.g. subcutaneous fluids, nutritional supplements such as high energy moist foods, gel foods, food treats etc.) to ensure they are remaining hydrated and receiving sufficient nutrition. If animals need to be anesthetized for imaging more than once in a 24-hour period, it may not be possible to fully recover animals in between sessions. These animals will need extra monitoring and supportive care. Animals may become hypoglycemic with repeated imaging. A drop of 5% dextrose solution diluted in LRS can be given by mouth or small amounts can be administered intra-peritoneally.

Specific Modality: MicroCT

High resolution x-ray computed tomography (MicroCT) produces detailed three-dimensional images of soft and hard tissues. Contrast agents are used to enhance x-ray attenuation. Radiation dose received by an animal or specimen in the micro CT is a function of the x-ray tube potential and current and the total time of exposure. For imaging that uses radiation, total cumulative amount of radiation an individual mouse can receive over the duration of the experiments should be calculated. Examples of exposures are shown in Table 2.

- **Typically, an individual mouse can receive 6.0 Gray (Gy, which is the unit of absorbed radiation dose of ionizing radiation) in total over the duration of experiments.**

Table 2: Example protocols and corresponding dose estimates are shown below * (Michigan State University http://research.rad.msu.edu/Facilities/index.html)

Potential (kVp)	Current (μA)	Exposure Time (ms)	Isotropic Voxel Size (μm)	Frames	Views	Field of View (mm)	Bed Positions	Dose Per Bed Position (Gy)	Total Dose (Gy)
80	450	200	92	2	720	32.8	1	0.25	0.25
80	450	200	92	2	720	98.4	3	0.25	0.76
80	450	400	92	2	400	65.6	2	0.28	0.56
80	450	400	92	1	360	98.4	3	0.13	0.38
80	450	400	92	1	720	98.4	3	0.25	0.76
80	450	400	92	2	360	98.4	3	0.25	0.76
80	450	400	92	3	360	98.4	2	0.38	0.76
80	450	400	92	4	360	98.4	1	0.50	0.50
80	450	200	92	2	720	98.4	3	0.25	0.76
80	450	200	92	3	360	98.4	3	0.19	0.57
80	450	200	92	4	360	98.4	3	0.25	0.76
80	450	800	46	2	200	98.4	3	0.28	0.84
80	450	1600	46	1	200	98.4	3	0.28	0.84
60	450	800	92	2	360	98.4	1	0.50*	0.50*

- Radiation dose measurements have not been performed at 60 kVp. The measurement at 80 kVp was used for these dosimetry calculations.

Affect of Radiation on Animal health

Be aware that radiation can affect animal health in unexpected ways. For example, in longevity studies, repeated *in vivo* scanning which exposes animal to ionizing radiation can result in unwanted effects to tissues or processes being studied. Adverse events that have been reported include:

- Reduction in bone density occurred when young mice were scanned (Klinck et al. 2008). Doses ranging from 2.5 to 8.0 Gy have been shown to disrupt the activity of osteoblasts and

osteoclasts (Laperre et al. 2011). Both these cell types are important for bone growth and health.

- Peritonitis (inflammation in the abdominal cavity) secondary to trauma during the IP injection of contrast agent.
- Nausea (manifested as generalized ill appearance) and/or diarrhea.
- Hematological disorders. Note that the damage disproportionately affects the gastrointestinal tract and the hematopoietic (blood) system due to the mitotic activity of these systems.
- Cell death
- DNA damage
- Cardiac hypertrophy (increase in amount of heart muscle), decreased left ventricular systolic function
- Retarded growth
- Opportunistic infections

Summary of Research on Radiation Exposure and Impacts on Rodent Health

"Mice can readily recover from whole body exposures of 0.25 to 0.5 Gy per day (Ford et al. 2003). The recovery rate is greater for partial body exposure and may be as much as 33% of the LD50/30 (the threshold at which 50% of the population die within 30 days after exposure) per day when only the lower body is exposed (Ford et al. 2003). However, continued exposure may damage the recovery process. The rate of life shortening has been estimated at 7.2%/Gy (Willekens et al. 2010). The LD50/30 for mice is 5.0 to 7.6 Gy, depending on age and on the resilience of the mouse strain to radiation exposure (Ford et al. 2003). C57BL/6 mice have been shown to endure 3 scans per week with an average dose of 0.28 Gy per scan over the course of 6 weeks for a total dose of 5.04 Gy without any significant effect on pulmonary or myocardial tissue (Detombe et al. 2013). Another study demonstrated that C57BL/6 mice could endure 3 scans of the hind limb with a dose of 0.434 Gy per scan with an interval of 2 weeks between scans without significant changes in bone structure, but 3 scans at 2 week intervals with a dose of 0.776 per scan induced some trabecular bone loss (Laperre et al. 2011).

In practice, due to the variability in the tolerance of different strains and individual mice to the effects of radiation, efforts to predict and prevent radiation-induced morbidity and mortality are imperfect. Therefore, daily monitoring is essential to ensuring humane treatment of the animals. " (Excerpt from Michigan State University IACUC SOP http://research.rad.msu.edu/Facilities/index.html)

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