WVU IACUC STANDARD OPERATING PROCEDURE:
Use of the Chronic Animal Monitoring and Surgical Facility (CAMF) – Chronic Instrumentation – RAT

1. Background

The Chronic Animal Monitoring and Surgical Facility (CAMF) provides telemetric implantation and analysis to West Virginia University researchers and their collaborators using equipment from Data Sciences International. Conveniently located within WVU’s Health Sciences Center, this facility performs chronic monitoring on small rodents (rats and mice). Animals, while under the care of the CAMF will be housed in an IACUC/OLAR approved room to recover from implantation and for data acquisition. The CAMF is a fee-for-service facility operated by a skilled and experienced technician who is readily available for consultation and assistance with experimental design, data acquisition and data interpretation.

Implantation of telemetric devices has become the gold standard for measuring heart rate, electrocardiography (ECG), blood pressure, temperature and activity in many species of animals and has opened up new areas of research. According to McCormick et al (2010), “…measurement of blood pressure via telemetry in a variety of laboratory animals has become an indispensable part of cardiovascular physiology, drug development and safety pharmacology.” The telemeters allow relevant data to be sent by radio transmission from the freely mobile, un-anesthetized animals to a remote receiver. The data can be collected in very specific intervals or continuously, allowing for comprehensive analysis of the various parameters.

All animals used in the CAMF will be under a WVU investigator’s IACUC-approved protocol and follow this SOP. The investigator’s protocol will provide a scientific justification for the implantation of telemetric devices, acquisition times, experimental groups, numbers of animals needed, assurances or justifications regarding unnecessary duplication of previous work, and length of the study. All appropriate CAMF staff members must be included on each animal protocol, and Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC) protocols, if applicable.

Telemetric monitoring is a critical research tool for studying small animal models of human disease. These studies, with the use of chronic mobile monitoring of disease development and progression, or disease recovery will increase research accuracy and reduce the number of animals used. The radio-telemetry devices have the ability to record the temperature, arterial blood pressure, bio-potential and activity of the implanted animal. Having the capability to acquire these measurements in a controlled environment in an un-anesthetized mobile animal provides many advantages to the investigator. The goal of the CAMF is to support translational research conducted by WVU investigators.
2. Surgical Implantation Procedure

Animal Preparation:

Rat:

All survival surgery will be done in accordance with the WVU IACUC policy on Rodent Surgery and Post-Operative Care as well as the Pain and Distress Policy. Rats will be weighed and anesthetized using an isoflurane (3-5%) induction chamber or an injectable anesthetic (see below). Sterile ophthalmic ointment will be applied to the eyes of the animal to reduce corneal desiccation. Once the rat is anesthetized, it will be closely shaved over its entire abdomen and upper chest. Animals will then be transferred to the surgical area and externally heated with a temperature controlled warming pad and heat lamp. The body temperature will be monitored with a rectal probe during procedures. Deep anesthesia will be maintained with isoflurane. The abdominal and upper chest areas will be disinfected with at least three alternating swabs of Betadine and 70% isopropanol in a circular motion starting in the center of the area. A sterile drape will be placed over the rat to maintain an aseptic surgical site and field. When approaching the end of the procedure one of the following injectable analgesics will be administered parenterally: buprenorphine (0.1 mg/kg s.c.) or buprenorphine SR (1.0 mg/kg s.c.) and carprofen (5 mg/kg s.c.), or bupivicaine (<2mg/kg) locally into the abdominal incision in order to ensure sufficient pain relief without suppressing respiration.

3. Surgical Procedure:

a. Abdominal Aorta: Blood Pressure/Temperature/Activity

Using sterile instruments a 3cm mid-line skin incision will be made below the xiphoid process to expose the abdominal muscles. Similarly the muscles will be split along the mid-line (linea alba) to expose the mesentery. Before incising the abdominal wall, it will be elevated to avoid nicking the underlying viscera. The mesentery will be gently retracted to the animal’s right side with a warm sterile saline soaked gauze pad to expose the abdominal aorta. The abdominal aorta just above the iliac bifurcation will be briefly occluded using traction from two lengths of 5-0 or 4-0 silk suture placed just below the renal artery and just above the iliac bifurcation. Using a 23G bent needle, a hole will be made in the aorta for the blood pressure catheter to be inserted. The tip of the catheter will be inserted into the lumen of the aorta and placed just below the renal arteries. The catheter will be glued into place with a small amount of cyanoacrylate glue (e.g. Vetbond). Surgical mesh will be used to further stabilize the site. The cranial then caudal sutures will be released slowly and the aorta will be checked for leaks. The telemeter body will be left in the peritoneum and the ECG leads will be externalized at this time, if applicable (see below). Prior to completely closing the abdominal cavity, 3 mL of warmed sterile saline will be infused into the peritoneum and the intestines will be gently massaged back into place. Afterward, the muscle incision will be closed with 4-0 absorbable suture. The skin will then be closed using 4-0 non-absorbable suture or wound clips (e.g. 35W) **NOTE THAT THERE MUST BE SPECIFIC INFORMATION LATER IN THE DOCUMENT AS TO THE REMOVAL OF WOUND CLIPS IF THEY ARE USED**. Any excess blood will be gently removed from the incision site with warm sterile saline and triple antibiotic ointment will be topically applied to the surgical site to aide healing. To prevent any infection, Baytril drinking water (2.27% in water at 0.5 ml/kg) will be started 2 days before surgery. If written justification is provided an investigator may choose to have animals receive Baytril-free water prior to surgery.

b. Subcutaneous ECG Lead Placement (if applicable)
If the transmitter used also has ECG capabilities the leads will be externalized to the subcutaneous after placement of the transmitter but prior to closing the muscle wall. The leads will be externalized through the abdominal wall for attachment to the subcutaneous muscle using a large bore needle. The needle will be passed through the abdominal wall on the rat’s left side and the red ECG lead will be placed in the lumen of the needle and gently pulled through the abdominal wall externalizing the lead at the subcutaneous level. The same will be done on the animal’s right side using the clear lead. To properly place the ECG leads, which are now subcutaneous on each side of the animal’s body, the red lead (+) will be placed at the level of the lowest rib and a 4-0 non-absorbable suture will be used to suture the lead securely to the muscle of the abdominal wall. The clear lead (-) will be positioned on top of the right pectoral muscle. Using a solid trocar, a subcutaneous tunnel will be made leading to the pectoral muscle and an open piece of tubing will be pushed over the trocar, and the trocar gently removed. The clear lead will then be fed into the tubing until it reaches the right pectoral muscle region. At the proper level a small incision in the skin will be made to allow placement of the lead wire to the subcutaneous pectoral muscle as stated above using a 4-0 non-absorbable suture. The small chest incision will be closed using 4-0 non-absorbable suture. Any excess blood will be gently removed with warm sterile saline and treated topically with triple antibiotic ointment.

4. Anesthesia

*Isoflurane* – Animals will be anesthetized by inhalation of isoflurane at 0.5-5% with oxygen and titrated to effect during the surgical procedure. Animals will first be anesthetized in an induction chamber and then moved for their surgical procedure after reaching a surgical plane of anesthesia.

*Ketamine/Xylazine* – Animals will be anesthetized by intraperitoneal injection of Ketamine/Xylazine cocktail. Depth of anesthesia will be maintained at 1/3 – ½ the original dose of Ketamine.

- Induction: 40-90 mg/kg Ketamine; 5-10 mg/kg Xylazine
- Maintenance: 13 mg/kg -45 mg/kg of Ketamine alone

*Yohimbine Reversal* – 2 mg/kg for rats as IP or SC injection to reverse xylazine effects

Gas anesthesia equipment allows inhalant anesthesia to be maintained during surgical sessions while a heated surgical board and heat lamp are used to maintain optimal body temperature. Rats are kept hydrated with 5 ml subcutaneous NaCl solution. Animals are monitored throughout the procedure and until they fully recover from anesthesia as demonstrated with near normal or normal body temperature and the ability to completely self-right themselves from dorsal recumbency.

Animals that will be anesthetized via injectable anesthetics for periods longer than 10 minutes will have eye lubricant applied to the eyes. In addition, eye lubricant will be applied to eyes of all animals maintained under isoflurane via nose cone.

5. Hair Removal

Hair removal will be necessary for all surgical procedures. The preferred method of preparing animals for procedures is to shave them with #10 blade clippers. For complete hair removal, the depilatory Nair™ (Carter-Horner, Montreal, Quebec) will be applied. Nair will be applied to the animal with a cotton-tipped swab or cloth and will be wiped off after 15 -60 seconds. Some strains may require a second application. The animal will then be wiped with a wet cloth to remove any excess Nair to prevent skin irritation.

6. Housing of animals during studies

*Post-Surgical Recovery* – After fully recovering from anesthesia, animals in the care of the CAMF staff will be maintained on an isopad for 24-48 hours to prevent bedding from sticking to the incision where soft food will be available on the floor of the cage for 24-48 hours. Also during the first 24 hours of post-surgical recovery, the animals cage may be
maintained half on and half off of a warming pad to stabilize the animals temperature. Animals will be monitored 2 or more times per day for 72 hours or until the animal is judged to be stable. These animals will be housed in a designated ACUC and OLAR approved satellite facility specifically approved for the CAMF. Animals will remain in the care of the CAMF staff until they have recovered normal circadian rhythm which may take up to 7 days post-surgery. This will be determined when the animal’s incisions are stable and the animal exhibits stable eating and drinking habits, maintains body weight, and shows relatively normal activity. If the animal is chewing or irritating its skin closures it may be necessary to apply an Elizabethan collar until sufficient healing has occurred. During this time the animal will be supplied with soft food on a petri dish on the floor of its cage and, if necessary, a heavy duty water reservoir.

**Data Acquisition** – Animals will be returned to an OLAR designated housing site for any data acquisition requested by the principal investigator. They will be moved to the CAMF acquisition room from the animals’ designated holding room for the duration of the requested data acquisition period. At the conclusion of the acquisition period the animals will either be euthanized or be moved back to the PI’s designated animal room, depending on what is stated in the IACUC approved protocol.

7. **Transfer of Animals to the Chronic Animal Monitoring and Surgical Facility**

Prior approval must be granted by the OLAR staff for transfer of animals into the CAMF. A transport cart must be used to avoid dropping a cage. The CAMF staff is responsible for transporting animals between the designated holding and procedure rooms. The wheels on the transport cart will be disinfected with a high- level disinfectant before entry into any rooms. All cages must be covered with a drape during transport from vivarium to procedure rooms.

8. **Personal Protective Equipment**

A disposable gown, mask, hair net and sterile gloves will be worn during any surgical procedure or before entering the CAMF holding/data acquisition room. Booties will be used when entering the holding room using the step in method of application and will be removed using the step-out process. An N-95 respirator and safety glasses can be worn, if needed. However, for each individual user the N-95 respirators must be fit tested by Occupational Medicine before use.

9. **CAMF Cleaning Procedures**

All surfaces that come into contact with the animal must be cleaned thoroughly and disinfected. Use OLAR approved disinfectant (Virkon®, Spor-kLens®, etc.) on non-sensitive surfaces such as the induction box and hood. All contact surfaces will be cleaned prior to beginning and after surgical procedures.

10. **CAMF Surgical Forms and Cards**

After the animals have recovered from anesthesia, the procedures and any observations will be documented on the surgery form and CAMF And a pink OLAR surgery card if the animal is to be returned to the vivarium. The forms/cards will list all relevant information such as animal identification, genotype (if applicable), anesthesia, procedure, pain management plan and all observations. In addition, they can list substances injected and location of injections. These forms will be placed in a binder in the facility and a CAMF surgical procedure card will be placed on the cage and will stay with the animal until the date of euthanasia.

11. **Injections**

Qualified personnel may inject medications, anesthetics, and/or experimental substances via one or more of the following routes as dictated by study needs.
• Intraperitoneal
• Intravenous via tail vein
• Intravenous via carotid or jugular (under general anesthesia only)
• Subcutaneous
• Intracardiac (under general anesthesia only)

12. Blood Collection

**Submandibular** – The rat is manually restrained and a stab incision is made with a 4-5 mm lancet into the cheek, approximately halfway between the ear and the mandible, with enough force to produce a small hole. Drops of blood from the stick point are collected into a tube. A blood volume equivalent to about 1.0% of the animal’s body weight is the maximum that will be drawn. Bleeding typically ceases automatically, but can be stopped by applying light compression with a gauze pad for approximately 5 seconds. Rats will be monitored after the procedure to ensure that bleeding has ceased. If multiple collections are necessary, no more than 1% of body weight may be drawn within a seven day period. Although more than 2 samples will require more than 7 days recovery, and the possible requirement of HCT testing and special blood replacement techniques and fluid (normal saline) administration for volume replacement.

13. Identification

CAMF will identify animals with ear punches or ear tags. This method of identification involves only momentary restraint as well as momentary pain. No anesthesia is required with skilled application of these methods of identification. If necessary the CAMF staff will apply EMLA cream.

14. Disposition of Animals Following Study / Transmitter Retrieval

Timing of euthanasia with regard to telemetry will be dependent on the length of the study and, therefore, will be dependent on the Principal Investigator’s protocol, experimental design and the health status of the animals.

*We will need EVERY animal back to retrieve our transmitters. Care MUST be taken when removing the transmitter in order to maintain the integrity of the catheter. Due to this limitation, we require that only a trained CAMF member be allowed to remove the transmitter. This can easily be done at or after the time of tissue harvest and at the convenience of the principal investigator.*

Animals will be euthanized according to the ACUC policy on pain, distress and humane endpoints after consultation with veterinary staff.

**Primary euthanasia methods:**

• Inhalation of CO2
• Anesthesia (isoflurane or injectable) overdose
• Cervical dislocation under anesthesia

**Secondary euthanasia (confirmation) methods:**

• Cervical dislocation
• Bilateral thoracotomy
• Dissection and removal of a vital organ

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References