WVU IACUC – APPROVED STANDARD OPERATING PROCEDURES (SOP):
Use of the Animal Models in the Rodent Experimental Stroke and Surgical Core (RESS)

1. Background

Animal models have and will continue to play a vital role in the study of mechanism(s) of human stroke, as well as in the assessment of potential drug and alternative therapies for prevention, treatment and recovery from stroke. The goal of the WVU Rodent Experimental Stroke and Surgical (RESS) core is to support the translational research projects of investigators at West Virginia University (WVU).

The RESS core provides state-of-the-art animal models for researchers and their collaborators at West Virginia University. Conveniently located in Biomedical Research Center (BMRC) Rm.141 and Rm.142, Office of Laboratory Animal Resources (OLAR) in the WVU Health Sciences Center, the RESS currently performs stroke related models in rodents. The RESS core is a facility run by the Core Director and trained personnel, who are readily available for consultation and assistance with experimental design and data interpretation. This SOP can only be used for rats and mice.

The RESS core provides the instrumentation, expertise, resources and training for the incorporation of animal models of stroke into the research of investigators in the RESS core and researchers in the greater WVU neuroscience and cardiovascular research community.

The following items are necessary to use the RESS core:

1) The animals will remain on the individual investigator's protocol, which references this SOP.

2) The investigator's protocol indicates rationale for requesting experimental stroke animals, experimental groups, and numbers of animals needed, assurances or justifications regarding duplication of previous work, anesthesia/analgesia, humane endpoints, category E justification, and length of the study.

3) All appropriate RESS core staff members ***must*** be included in each animal protocol in order to provide this service.

4) The RESS core staff will train personnel on using RESS core services to prepare animals ready for surgery and provide post-surgery care.

This SOP will provide general surgical procedures listed below. The basic procedures can be placed into the investigator's animal use protocol, and deviations from the outlined procedures ***must*** be communicated with the stroke core staff to avoid non-compliance:

A. **Detection of Cerebral blood flow by Laser Speckle Imaging (LSI) or Laser Doppler Flowmetry (LDF)**
B. **Transient Middle Cerebral Artery Occlusion (tMCAO) Model**
C. **Permanent Middle Cerebral Artery Occlusion (pMCAO) Model**
D. **Distal Middle Cerebral Artery Occlusion (dMCAO) Model**
E. **Photothrombotic Stroke (PST) Model**
F. **Epidural Application (EA) Model**
G. **Ameroid Constrictor Arterial Stenosis (ACAS)**
H. **Bilateral Carotid Artery Stenosis (BCAS)**
I. **Traumatic Brain Injury (TBI)**
J. **Controlled Cortical Impact (CCI)**
K. **Microembolism**
L. **Subarachnoid hemorrhage**
M. **Non-survival Surgery for Physiological Measurements**
N. **Non-survival Surgery for Cerebrospinal Fluid Collection**

2. General Surgical Procedures

The following general protocol will be followed for animal models used in the RESS core. The details are limited to RESS core provided procedures as individual experiments are described in each investigator's approved protocol. Surgical procedures will adhere to the IACUC policy "Surgery Guidelines for Rodents". And all other IACUC policies will be adhered to unless justification is approved by the IACUC to deviate from these by the PI on their protocol.

2.1 Animal preparation

- The animals will be transferred to BMRC Rm.141, Rm. 142, or an approved procedure room in the vivarium at least one hour before the surgery.

2.2 Surgical preparation

- All surgical tools will be sterilized by autoclaving. We sanitize the surgery table and associated equipment using Peroxigard (or equivalent disinfectant) and sterilize instrument tips via glass bead sterilizers. All surgery will be conducted under aseptic conditions.
- The surgeon wears surgical gown, surgical mask, bouffant cap and sterile gloves. No food or drink is allowed in Pre-Op areas.

2.3 General Surgical Procedures

Pre-operative preparation for all surgical procedures:

- Surgical anesthesia will be induced with 4-5% isoflurane until the animal shows no response to toe pinch. Induction chambers ***must*** be flushed with air for a minimum of 10 seconds before opening. Anesthesia is maintained with 1-2% isoflurane via facemask in O_2-enriched air during surgery.
- After placing the animal on a heating pad, it will be fixed to the surgical table using adhesive medical tape on the tail and paws. For procedures requiring the use of the stereotaxic apparatus, the animal will be fixed to the apparatus using the ear and nose bars and will receive heat support during the procedure via the integrated warming base.
- Veterinary eye lubricant will be used on the animal's eyes to protect and lubricate eyes during anesthesia and surgical procedures.
- Surgical sites will be freed of hair by either clipping or application of a depilatory cream, to be applied for ~30 seconds and wiped/rinsed off thoroughly. The surgical site will then be disinfected using betadine scrub alternating with alcohol 3 times using sterile swabs.
- *Local analgesics (type, location, administration route, and dosing) will be determined by the individual lab's approved IACUC protocols.*
- *All other analgesics (i.e. additional systemic analgesics) will be given as determined by the individual lab's IACUC protocol recommendations.*

A. Detection of Cerebral blood flow by Laser Speckle Imaging (LSI) or Laser Doppler Flowmetry (LDF)

Surgery Classification: Minor, Pain severity: Mild, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- For using an LSI to detect brain blood flow, a 1-2 cm midline incision on the scalp beginning between the eyes and terminating caudal of the ears will be created using a scalpel to expose the skull. For using an LDF, a 1 cm incision between eye and ear will be made and a fiberoptic probe will be attached to the skull.
- The scalp will be retracted to the side of the head and bleeding will be stopped with sterile swabs or gauze pads.
- Animals will be placed under an LSI or LDF to measure the cerebral blood flow during which animals will remain adequately anesthetized. *The frequency of measurements will be determined by the individual lab's experimental needs.*
- The incision will be closed with sterile nylon sutures or surgical staples.

B. Transient Middle Cerebral Artery Occlusion (tMCAO) Model

Surgery Classification: Major + minor multiple survival surgical procedure, Pain severity: Mild, Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- A midline ventral neck incision will be made, and unilateral middle cerebral artery occlusion (MCAO) will be performed by making an incision (about 0.02 mm for mice and 0.04 mm for rats) and inserting a sterile nylon filament (*the occluder size is determined by individual lab's experimental needs and animal body mass*) into the internal carotid artery via an external carotid artery stump. The filament will be anchored by a sterile suture.
- The incision will be closed with sterile nylon sutures. At this point, the animal will be returned to a clean cage and allowed to emerge from anesthesia. *Depending on the protocol, the occlusion will last between 30 min and 120 min*. The surgeon is present during the entire procedure.
- The animal will be re-anesthetized to remove the occluder. The suture will be carefully removed, and the filament will be withdrawn, reestablishing circulation to the middle cerebral artery. After the removal of the filament, the external carotid artery will be permanently closed using an electrocauterizer.
- The skin incisions will be closed with sterile nylon sutures.
- Sham animals will undergo the identical procedure up to exposing the arteries, but no occlusion will take place.
- Ischemia/reperfusion may be verified by an LSI or LDF (detailed in General Surgical Procedures A).

C. Permanent Middle Cerebral Artery Occlusion (pMCAO) Model

Surgery Classification: Major, Pain severity: Mild, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- A midline ventral neck incision will be made, and unilateral middle cerebral artery occlusion (MCAO) will be performed by making an incision (about 0.02 mm for mice and 0.04 mm for rats) and inserting a sterile nylon filament (*the occluder size is determined by individual lab's experimental needs and animal body mass*) into the internal carotid artery via an external carotid artery stump. The filament will be anchored by a sterile suture.
- The incision will be closed with sterile nylon sutures.
- Sham animals will undergo the identical procedure up to exposing the arteries, but no occlusion will take place.

  Ischemia may be verified by an LSI or LDF (detailed in General Surgical Procedures A).

D. Distal Middle Cerebral Artery Occlusion (dMCAO) Model

Surgery Classification: Major, Pain severity: Mild, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- A midline ventral neck incision will be made, and the one common carotid artery (CCA) will be gently exposed with blunt forceps
- (*optional*) A slip knot will be tied around the CCA with sterile 6-0 coated ethilon suture
- An "L-shaped" skin incision will be made to expose the temporal bone overlying the middle cerebral artery. The horizontal incision will be made between the ear and eye (~4mm), and then a second cut vertically (~4mm) toward the parietal bone. The skin flap will be gently pulled back and retracted with blunt forceps, the temporal muscle will be carefully dissected using fine curved forceps and gently retracted to expose the temporal bone. The muscle will be incised and separated along natural fascial planes with care to avoid tearing, and the middle cerebral artery (MCA) visualized below the skull
- A micro drill will be used to make a burr hole in the skull at low speed. Drilling will be performed intermittently with irrigation using sterile saline applied through to prevent thermal injury to underlying tissues. Drilling will be stopped upon reaching the dura and the final bone layer will be gently lifted away using fine forceps.
- Once the MCA branching is exposed:
    - *If permanent dMCAO is desired:* a 6-0 nylon suture will be passed gently under the MCA branch to elevate and stabilize the vessel, a fine-tip bipolar microcautery will be used to coagulate the MCA branch. The surgical field will be kept moist with sterile saline. To prevent recanalization the vessel will be cauterized and then sharply transected at the center of the cauterized segment using fine vannas scissors.
    - *If transient dMCAO is desired, 2 options are listed below:*
        - A nylon suture will be tied loosely around the MCA branch and a microsurgical needle placed parallel to the vessel. The knot will be tied against the needle shaft. At the end of the occlusion period, the needle will be slowly withdrawn, allowing the loop to loosen and the suture to be removed
        - Alternatively, a fine microvascular clip will be placed across the MCA branch for the desired occlusion time and gently released to allow reperfusion
        - *duration of occlusion will be determined based on experimental needs*
- The temporal muscle will be gently repositioned over the burr hole and skin will be gently pulled back into place and closed with suture.
- (*If the CCA was occluded*) The knot around the CCA will be released and neck incision will be closed with suture.
- Ischemia may be verified by an LSI or LDF (detailed in General Surgical Procedures A).

- Sham procedures will be tailored to the experimental needs of the PI. The default sham procedure will involve animals undergoing the identical procedure up to skin and muscle retraction but no drilling or cautery will take place. If it is necessary to control for the effects of the skull drilling, sham animals will undergo the full surgical procedure up to drilling, but the bone will thinned rather than removed and the vessels will not be disturbed.

E. Photothrombotic stroke (PTS) Model

Surgery Classification: Minor, Pain severity: Mild, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- To prevent off-target effects of ambient lighting, a sterile drape will also be positioned over the animal with an opening at the incision site (see below). This will protect the eyes and skin from potential damage caused by ambient light in the room.
- Rose Bengal will be administered prior to surgery. *The dose and route of administration of Rose Bengal will be determined based on experimental needs of individual labs* (less than 200mg/kg, 300µl of sterile solution for injection).
- The mouse will be fitted onto the stereotaxic apparatus.
- A 1-2 cm midline incision on the scalp beginning between the eyes and terminating caudal of the ears will be created using a scalpel to expose the skull.
- A cold light source (KD 2500 LED) will be used to induce ischemia for 10-30 min (*depending on targeted severity)* using stereotaxic coordinates *as determined by the experimental needs of the individual lab*. A dose of 5000 lux is delivered which results in a light dose of 1.3 W/cm^2 or 1170 J/cm^2.
- Sham animals will either receive the same dose of Rose Bengal but not undergo light exposure or will receive a vehicle injection followed by light exposure. *The sham procedure will be determined by the individual labs based on experimental needs.*
- As an additional precaution to reduce off-target effects of ambient light, the cage will be maintained in a dark chamber or dark room for a minimum of one hour following surgery.
- Ischemia may be verified by an LSI or LDF (detailed in General Surgical Procedures A).

F. Epidural Application (EA) Model

Surgery Classification: Major, Pain severity: Moderate Analgesic Recommendation: Pre-emptive + 24 hours post-op, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- The animal will be fitted onto the stereotaxic apparatus.
- A 1-2 cm midline incision on the scalp beginning between the eyes and terminating caudal of the ears will be created using a scalpel to expose the skull.
- The fascia will be carefully removed from the skull using fine-tipped forceps.

- Using a dental drill or a sharp blade, an area of the skull approximately 0.5-2mm in diameter will be thinned until the skull cracks, dividing the thinned area into several segments.
- Throughout the procedure, the skull will be kept moist with drop applications of sterile saline.
- The craniotomy will be performed by gently removing the thinned skull segments using extra-fine tipped forceps.
- Drug(s) will be administered topically (*as approved in PIs' IACUC protocol*).
- The incision will be closed with a sterile nylon suture.

G. Ameroid Constrictor Arterial Stenosis (ACAS)

Surgery Classification: Major, Pain severity: Mild, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- Under a surgical microscope, a midline skin incision (0.5 cm) will be made on the neck, and both common carotid arteries (CCA) will be exposed and freed from their sheaths.
- A sterile (via ethylene oxide gas or low temp hydrogen peroxide plasma sterilization) ameroid constrictor ring will be placed around the exposed right common carotid artery.
- A sterile microcoil will be placed on the left CCA.
- Sham animals will undergo the identical procedure up to exposing the arteries but neither the constrictor nor microcoil will be placed around the arteries.
- The incision will be closed with a sterile nylon suture.

H. Bilateral Carotid Artery Stenosis (BCAS)

Surgery Classification: Minor, Pain severity: Mild, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- Under a surgical microscope, a midline skin incision (0.5 cm) will be made on the neck, and both common carotid arteries (CCA) will be exposed and freed from their sheaths.
- A sterile microcoil will be placed on each CCA.
- Sham animals will undergo the identical procedure up to exposing the arteries but microcoils will not be placed around the arteries.
- The incision will be closed with a sterile nylon suture.

I. Traumatic Brain Injury (TBI)

Surgery Classification: Minor or Major (depending on degree of impairment), Pain severity: Mild to Moderate depending on impact, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or Pre-emptive + 24 hours analgesia, local + NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- The animal will be fitted onto the stereotaxic apparatus.
- A 1-2 cm midline incision on the scalp beginning between the eyes and terminating caudal of the ears will be created using a scalpel to expose the skull.
- The skull impactor will then be carefully positioned over bregma and moved to the desired coordinates (*the stereotaxic coordinates will be determined by the experimental needs of the PI*)
- The impactor will be retracted and driven into the skull. *Note: the tip diameter, impact velocity, and dwell time will all be determined by the experimental needs of the PI. These variables will determine the intensity (mild, moderate, severe) of the TBI.*
- Sham animals will undergo the identical procedure, but the impactor tip will be gently lowered onto the skull and retracted.
- The skin incision will be closed with sterile nylon sutures or surgical staples.

J. Controlled Cortical Impact (CCI)

Surgery Classification: Major, Pain severity: Moderate Analgesic Recommendation: Pre-emptive + 24 hours post-op, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- The animal will be fitted onto the stereotaxic apparatus.
- A 1-2 cm midline incision on the scalp beginning between the eyes and terminating caudal of the ears will be created using a scalpel to expose the skull.
- A 3-4mm craniotomy will be performed in the parietal bone with a high-speed drill and the bone flap will be carefully removed with fine tipped forceps.
- The tip of the impactor will then be carefully centered above the craniotomy, it will then be retracted and driven into the dura. *Note: the tip diameter, impact velocity, and dwell time will all be determined by the experimental needs of the PI. These variables will determine the intensity (mild, moderate, severe) of the CCI.*
- Sham animals will undergo the identical procedure which may include the craniotomy *(depending on the experimental needs of the individual lab)* but the impactor tip will be gently lowered onto the skull and retracted.
- Hemostasis will be restored with gentle pressure using sterile gauze and the skin incision will be closed with sterile nylon sutures.

K. Microembolism

Surgery Classification: Major, Pain severity: Mild, Analgesic Recommendation: Pre-emptive, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- Under a surgical microscope, a midline skin incision (0.5 cm) will be made on the neck, and a sterile polyethylene tube (PE-10) will be inserted into a unilateral internal carotid artery (ICA) and sterile microspheres suspended in saline will be injected in a maximum of 100µL. *The number and diameter of spheres will be determined by the experimental needs of the PI.*
- Following the injection, the polyethylene tube will be withdrawn, gentle pressure and Gelfoam will be used to maintain hemostasis, and the skin incision will be closed with sterile sutures.
- Sham animals will undergo the identical procedure but only saline will be injected into the ICA.

L. Subarachnoid hemorrhage

Surgery Classification: Major, Pain severity: Moderate Analgesic Recommendation: Pre-emptive + 24 hours post-op, Local +/- NSAID or opioid

- Animals will be prepared for surgery as outlined above under "Pre-operative preparation for all surgical procedures"
- Under a surgical microscope, a midline skin incision (0.5 cm) will be made on the neck, and a unilateral common carotid artery (CCA) will be exposed.
- An incision will be made into the internal carotid artery (ICA) and a sterile nylon filament (0.2mm diameter) will be advanced into the ICA until resistance is encountered, then advanced an additional 1-2mm to ensure perforation of the vessel wall at the bifurcation of the anterior and middle cerebral arteries.
- The suture will then be retracted, and the surgical incision will be closed with sterile sutures.
- Sham animals will undergo the identical procedure but the ICA will not be cut.

M. Non-survival surgery for physiological measurements

Surgery Classification: Non-survival, No analgesics provided.

- Each animal will be prepared as for the survival experiments, however clean but not sterile technique will be employed. Surgical anesthesia will be induced with 4-5% isoflurane until the animal shows no response to toe pinch and maintained with 1-2% isoflurane via face-mask in O_2-enriched air.
- The femoral artery will be cannulated for measurement of arterial blood gases, blood pH, and blood glucose, and blood pressure.

- The skin over the cranium will be opened, and a 1 mm area of lateral parietal bone will be thinned, and a small fiberoptic probe will be positioned over the thinned area.
- Cerebral blood flow will be detected by LDF (detailed in General Surgical Procedures A).
- Repeated measurements of arterial blood gases, blood pH, blood glucose, and blood pressure, and brain blood flow will be recorded as needed. At the end of the experiment, isoflurane will be increased to 5% and the animal will be euthanized.

N. Non-survival surgery for cerebrospinal fluid collection

Surgery Classification: Non-survival, No analgesics provided.

- Each animal will be prepared as for the survival experiments; however clean but not sterile technique will be employed. Surgical anesthesia will be induced with 4-5% isoflurane until the animal shows no response to toe pinch and maintained with 1-2% isoflurane via face-mask in O_2-enriched air.
- Hair will be removed from the back of the neck using a depilatory cream which will be on the skin for 30 seconds, then wiped off and the skin rinsed with water.
- An incision will be made in the skin and underlying muscle will be blunt dissected to expose the dura over the cisterna magna
- The tip of a pulled glass capillary tube will be inserted at the midline just caudal to the occipital bone to puncture the dura in the cisterna magna and ~8-15µL of cerebrospinal fluid will be collected through capillary action
- At the end of the collection, isoflurane will be increased to 5% and the animal will be euthanized.

2.4 Post-surgical care

Once returned to OLAR, animals will undergo post-surgical monitoring and follow-up as defined in the IACUC policy: Surgery Guidelines for Rodents. All humane endpoints will be determined by the individual IACUC-approved protocols. In addition, we added essentials for post-surgery care as below:

1) *All postoperative analgesia procedures will be determined by the individual lab's approved IACUC protocols.*
2) The animals will be monitored until they are stable, and at least 60 minutes after surgery, even if the animals are stable and fully out of anesthesia. This monitoring will be documented in the lab's records. The animals will be housed for observation and additional pain control as needed in room 142 of the RESS core. Cages will be partially placed on a heating pad (one side of the cage will remain off the heating pad so that the animals may choose to move away from the heat source if it causes discomfort). Only animals fully emerged from anesthesia, will be returned to the vivarium within 23 hours.

3) All animals will be monitored for food and water intake. If necessary, subcutaneous fluids or HydroGel may be given *per IACUC-approved protocol*. Mice undergoing procedures that may produce mobility issues will be provided with soft bedding. New nesting material will also be provided on the day of surgery.
4) Animals will be monitored for signs of pain daily using general appearance, posture, body condition, respiration, body temperature and locomotion. All data, including overall recovery scores will be recorded daily as indicated on pink surgery cards by the PI lab's staff/students. Animals will be weighed and observed for signs of dehydration. If needed, animals will be given subcutaneous fluids (i.e. saline).
5) Cages containing animals undergoing treatment for pain or dehydration will be clearly marked. However, if an OLAR animal care giver or veterinary staff member finds an animal that is exhibiting signs of pain (grimace, hunched posture), infection, or dehydration they will call the PI's lab (refer to individual IACUC protocol) to take care of the animal.

Humane Criteria for euthanasia

Our models produce a neurological injury; while pain and distress are not commonly observed, animals will be monitored carefully for the following:

1) Wound ulcers are rarely observed and will result in euthanasia.
2) Wound frank bleeding may occur if sutures are removed by the mouse, and may result in euthanasia based on endpoints in the PI's protocol.

Note, this is not a complete list of endpoints, endpoints need to be determined on a protocol-specific basis based on the needs of the study and the model (combination of models) used. Additional endpoints may include 20% weight loss from baseline, ambulation, inability to reach food/water, and lethargy that does not improve within a pre-determined period of time.

Post-surgical monitoring sheets will document these changes and will be combined with records for surgeries performed, anesthesia and analgesia administered, and any complications encountered. If any of the above signs are observed, the RESS core staff member will be notified and veterinary advise regarding humane endpoints will be followed. However, some animals will have conditions/symptoms (i.e. circling due to unilateral ischemic damage to brain regions that control motor function), which is a normal progression of the ischemic insult and will not be treated. If vet staff deems pain or distress to be too significant or intractable, the animal will be euthanized.

3. Housing of animals during studies

Animals will be housed in the OLAR vivarium rooms designated for holding animals involved in stroke animal studies.

4. Transfer of Animals to RESS core

The RESS core staff and PIs' lab is responsible for transporting animals between the designated holding and surgical operation rooms in the RESS core. The wheels on the transport cart will be disinfected with Peroxigard (or equivalent disinfectant) before exit and entry into any rooms. All cages will be covered with a drape during transport.

5. Personal Protective Equipment (PPE)

A disposable gown, mask, hair net and gloves will be worn for all animal work. Gloved hands are to be kept clean during animal handling procedures. When exiting, remove all PPE.

6. RESS core Cleaning Procedures

All surfaces that come into contact with the animal ***must*** be cleaned thoroughly. Use Peroxigard on the surgical operation table and anesthesia induction box. Surfaces will be cleaned prior to beginning a surgical procedure, between cages of animals and after the completion of surgery.

7. Surgical Procedure Sheets

After the animals have recovered from anesthesia, the procedures and any observations will be documented on the pink surgery/anesthesia card. Surgical procedure sheets will be used for surgical record procedures. The sheets will list dates of surgery, procedures, observations and the initial of surgeons. In addition, they will list substances injected and location of injections.

8. Injections

Qualified personnel will inject medications, anesthetics, analgesics, and/or experimental substances via one or more of the following routes as dictated by study needs.

- Intraperitoneal
- Intravenous via tail vein
- Intracerebral injection (under general anesthesia only)
- Subcutaneous
- Intracardiac (under general anesthesia only and only for non-survival procedures)

9. Identification

Tail tattooing may be performed using the AIMS (animal identification & marking systems) or ear

tags. Animals will be tagged while under anesthesia for the above procedures. The identification information will be documented on surgical procedure sheets and ***must*** be described in your IACUC protocol.

10. Tail Biopsy

RESS core staff will perform tail biopsies on mice and rats before 17 days of age. Less than 3 mm of the tail will be removed. The scalpel will be disinfected between animals. The IACUC policy "Tail Biopsy for DNA Extraction in Mice" will be followed. This procedure ***must*** be described in your protocol.

11. Sample Collection Following Study

The timing of euthanasia will be dependent on the length of the study and, therefore, will be dependent on the Principal Investigator's protocol and the health status of the animals.

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

Primary euthanasia methods:

- Inhalation of CO_2
- Anesthesia overdose
- Cervical dislocation under deep anesthesia

Blood Collection, Dissection and removal of vital organs,

- For survival animals, the optional sample bleeding methods as follows: Superficial Temporal Vein Sampling (limited to adult mice and rats), Submandibular Blood Sampling in Mice (2010).); Saphenous Sampling (medial or lateral approach, (http://film.oslovet.veths.no/saphena/ and Journal of Visualized Experiments. Online video. http://www.jove.com/index/details.stp?id=266); Lateral Tail Vein or Ventral/Dorsal Artery Sampling; Tail nick Sampling (J Am Assoc Lab Anim Sci. 2008 May;47(3):8-15.).
- For non-survival surgery: The blood samples will be collected once via cardiac puncture or cannulated femoral artery under terminal anesthesia at the end of experiments; Animals will be dissected, and other organs will be collected once the animals are euthanized by decapitation under deep anesthesia.

12. RESS Core Personnel

Ekaterina Weil Ph.D., Director of the RESS core

Liz Liu M.D., Core Surgeon

Tara Craft Ph.D., Core Surgeon

Debbie Corbin, B.S., Core Technician

13. RESS Core Contacts

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