Exhibit 17

17) July 21, 2013 letter from UW IO providing information on the implementation of the recommendations.
    a) Eye & ear coil maintenance standard operating procedure (SOP)
    b) Non-surgical procedures: No anesthesia SOP
    c) Sanitization of cat socialization and behavioral training room SOP
    d) Animal transportation SOP
    e) Animal equipment cleaning SOP
    f) Explant maintenance SOP
    g) Non-surgical procedures: Anesthesia required SOP
    h) Copy of amended protocol dated 8/26/13 submitted subsequently

Graduate School
UNIVERSITY OF WISCONSIN–MADISON

August 21, 2013

Dr. Axel Wolff, MS, DVM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
RKL1, Suite 360
6705 Rockledge Drive, MSC 7982
Bethesda, MD 20892-7892

Dr. Wolff:

This letter provides a progress report of our efforts to address findings and recommendations detailed in your letter of May 7, 2013 following your visit to the University of Wisconsin-Madison on April 17th and 18th.

Following extensive veterinary pre-review, the revised animal care and use protocol underwent full committee review at the August 5th meeting of the School of Medicine and Public Health (SMPH) ACUC. While conducting the review, the Committee carefully weighed the potential adverse events to an individual animal against the potential scientific, human clinical, and societal value of the research. The Committee voted to require modifications to secure approval. This process is ongoing, and I will forward the protocol to you as soon as it is approved.

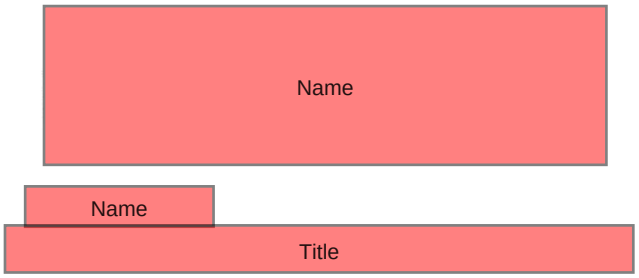
Also at the August 5th meeting, the SMPH ACUC reviewed and approved new and amended laboratory-specific SOPs (attached). These were written through collaborative efforts of the laboratory and veterinary staffs, and Research Animal Resources Center trainers.

Laboratory Animal Resources (LAR) leadership, working with veterinary and laboratory staff, identified a room within the animal barrier to serve as a dedicated, sanitizable, and secure area for human-animal socialization and behavioral training activities that are utilized by the

laboratory. A consultation with a veterinary infectious disease specialist from the School of Veterinary Medicine was performed to assist in identifying an appropriate room and developing best practices for use of the room. Use of the room was discussed and approved in August by the SMPH ACUC. An LAR SOP describing maintenance of the room is attached. The infectious disease specialist will be available for further consultation in the future if needed.

Please let me know if you require any further information at this time.

Best Regards,



Attachments:

Laboratory SOPs regarding:

- Explant maintenance
- Eye and ear coil maintenance
- Animal transportation
- Food preparation and equipment maintenance
- Non-surgical procedures: anesthesia required
- Non-surgical procedures: no anesthesia required
- Animal equipment cleaning

LAR SOP regarding maintenance of the socialization and behavioral training room.

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Tuesday, August 27, 2013 8:20 AM
To: Name
Subject: RE: UW-Madison protocol

Thanks very much, Name We'll go over all these documents carefully and will be in touch as to the next steps.
Axel Wolff

-----Original Message-----
From: Name [mailto: Name @wisc.edu]
Sent: Monday, August 26, 2013 2:19 PM
To: Wolff, Axel (NIH/OD) [E]
Subject: UW-Madison protocol

Dr. Wolff,
Please find attached an approved protocol for your consideration.
Please let me know if your require further information or have any questions.
Sincerely,
Name

Eye & Ear Coil Maintenance
Standard Operating Procedure

University of Wisconsin Madison
Yin Lab

SOP# 2 Date Revised: 7/10/13

Location(s)Room #Medical Science Building
Responsibility: Yin Lab Staff
Safety: Personal Protective Equipment (PPE)

I. Purpose: Procedure to fix the eye or ear coil if it is partially pulled out at wound edge, is visible in the eye, or is broken.

II. Materials Needed:

0.9% sterile saline solution (1000 ml bottle)
Sterile cotton swabs
Sterile gauze
Syringes (1cc–3cc)
Needles (16g–23g)
Alcohol swabs or bottled alcohol applied with sterile gauze.
Yin Lab Daily Animal Log
Anesthesia Log, top page
Anesthesia Log, extra pages
Sterile ointments to be used in eye:
- Artificial tears
- TAO Triple Antibiotic Ointment (ophthalmic)
- Other antibiotics, per RARC vets

Instruments (stored in cold sterilization tray (Cetylcide G)
- Scissors (2)
- Forceps (2)
- Eye-lid retractor (2)
- Sterile 7-0 silk suture with needle attached.

III. Documents/Attachments:
Yin Lab Daily Animal Log
Anesthesia Log, top page
Anesthesia Log, extra pages

IV. Safety:

1. All personnel handling these specified animals must be adequately trained in methods of aseptic technique.
2. PPE including clean gloves are worn and applies to each section of this SOP. Wash hands prior to and after donning or doffing gloves.
3. A face mask must be worn.
4. Put the animal in a restraint bag.

V. **Directions:**

A. Determine course of action:

1. If the coil is pulled out at the wound edge, carefully examine the eye and tissues of the eye, cheek and ear, to see if there is any trauma or tension on the tissues.
2. Determine exactly which coil is involved by referring to coil placement documentation in the surgical record and by measuring the coil resistance with the ohm meter.
3. If the coil measures in an acceptable range, consult Dr Yin about whether the coil placement can be repaired. This may involve partially reshaping the coil and resecuring it to the eye ball or pinna (see item B), or using dental cement to bury exposed coil.
4. If the coil is no longer functioning but there is no tension or trauma on tissues and no exposed wires, simply document the coil failure on the "cat connector Map" located in the Yin Lab, and in the cat's lab notebook.
5. If the coil cannot be repaired and is causing trauma to tissues, remove it as described in item B.
6. If it is not causing trauma to tissues, but is exposed at the wound edge, cut exposed wire away with scissors.
7. Document any coil removal and wire cutting in the medical record, the "cat connector map" and the "Cat surgery info log" located on D drive of the catscan computer.

B. Procedure for repairing or removing broken or exposed eye/ear coil under local anesthesia:

1. Only Tom Yin or persons trained and authorized by him will repair or remove broken eye/ear coils.
2. Notify vet staff by e-mail or telephone that a procedure to repair or remove broken or exposed eye/ear coil will need to be performed.
3. If possible, delay procedure until the animal has fasted 12 hours. Put the fast sign on the door and write on the white board on 6th floor a day before the procedure. If coil removal cannot be delayed (i.e. there is the potential of damage to the eye or surrounding tissue) consult vet staff immediately.
4. Sterile instruments are maintained at all time in cetylcide G in a cold sterilization dish in the lab. Soak needed sterile instruments in sterile water once they are retrieved from the cetylcide g solution. Document

in the Anesthesia log and extra log pages. Document in the medical record.

5. Anesthetize the cat and monitor animal vital signs per protocol M00212.
6. If necessary, remove eye coil.
   a. Put artificial tears in unaffected eye.
   b. Place the sterile eye piece in the eye under the third eye-lid, to hold the eye open.
   c. Eye coil is removed at the eye. Hold the eye coil securely with two forceps at two adjacent positions. Cut the coil between the two forceps with a scissors. Gently pull on each forceps to remove the coil with care to remove all pieces of the coil.
   d. When procedure is complete, put TAO ophthalmic in affected eye.
7. If able, repair eye coil.
   a. Put artificial tears in unaffected eye.
   b. Place the sterile eye piece in the eye under the third eye-lid to hold the eye open.
   c. Use 7-0 suture to secure the eye coil to the eye at the necessary locations.
   d. When procedure is complete, put TAO ophthalmic in affected eye.

Approved by: Name 8/9/13
Name, Senior Program Veterinarian Date

Approved by: Jon C. J. S 8/13/13
Name, Supervisor Date

Non-Surgical Procedures: No Anesthesia
Standard Operating Procedure

University of Wisconsin Madison
Yin Lab

SOP# 6 Date Revised: 7/10/13

Location(s Room # Medical Science Building, animal housing area.
Responsibility: Yin Lab Staff
Safety: Personal Protective Equipment (PPE)

I. Purpose: To safely administer injections, ointments, and oral medications per protocol or vet order.

II. Materials Needed:
Yin Lab Daily Animal Log
Animal medical record
Sterile syringes (variety from 1cc to 5cc)
Sterile needles (23 gauge)
Sterile cotton swabs
Medications

III. Documents/Attachments:
Yin Lab Daily Animal Log

IV. Safety:
PPE including clean gloves and lab coat.

V. Directions:

A. **Do not give any medications unless you have been trained and approved to do so.** Always double check that you are using the correct medication and the correct dosage. Never use expired drugs.

B. Injections

1. Sub-cutaneous injections. Make sure the cat is properly restrained. Scruff a portion of loose skin on the upper back between the shoulder blades. Insert the needle at the base of the tented folds of skin. Pull back on the plunger of the syringe to verify that a vacuum is created, then inject the medication.
2. Intramuscular injections. Always use a separate sterile needle for drawing up the medications, and a new needle

for injecting the cat. Wipe the top of the bottle with alcohol. Withdraw the calculated dose carefully making sure there is no air in the syringe. A second person will be needed to properly restrain the cat. Once the cat is restrained, the individual giving the injection will grasp a hind leg and pull it straight a bit. Palpate the muscle where the injection will be made to ensure it is not close to a bony area. Insert the needle, draw back on the plunger to verify that a vacuum is created and to ensure a vein or artery has not been struck. If blood is visible, withdraw the needle and syringe, place them in the sharps container and start over. If no blood is visible and the vacuum is created, proceed and inject the medication into the muscle, making sure the cat gets the entire dose.

C. Eye ointment
   1. Use exam gloves and sterile cotton swabs to apply eye ointment. Never touch the tip of the tube to anything, especially not the animal. Squeeze the eye ointment on to the swab without the swab touching the tube. Ensure the animal is properly restrained so the eye is not injured during the application of the eye ointment. The eye ointment is applied without touching the swab to the eye.

D. Oral medications
   1. Cephalexin and Cephazolin are kept in the refrigerator. Both will need to be mixed per manufacturers directions before administration. Use sterile water to mix the medications and follow directions carefully. Label the bottle with the concentration, date of expiration and initials of the person mixing the medication.
   2. Medication is administered either with a small syringe orally, or mixed in a small amount of soft food. Make sure the cat receives the entire dose by insuring the syringe is empty, or insuring that the cat has eaten all the food and medication.

E. Documentation
   1. Be sure to document the subject's correct name and number into the corresponding medical record. Be sure to document all appropriate activity with the animal into the medical record. Never pre-date, post-date or intentionally falsify records.
   2. Use Black Ink.
   3. Always document the time and date of the entries. Initial or sign your entries.
   4. Document drug dosages in ml as well as total mgs. (Different drugs can have different concentrations. The total amount of mgs is what is important.)

5. Only use approved abbreviations. (They are listed on a poster in the charting area.)
6. If you make an error (write in the wrong record or write incorrect information), strike the incorrect entry will a single line (so it can still be read), and write "error" and initial.
7. If you do a treatment but forget to document it, you need to make a "late entry". Date the entry the day you write it, for example 12-10-12. Write "late entry for 12-08-12, .62 mgs (1.6)ml cephalexin given in moist food. Cat ate all medication. Include signature or initials.
8. Document mode of administration, subQ, IM, oral, in moist food etc. Document if the cat finished the med if it was given in food.
9. If you forget to give a medication, notify the vet staff immediately

Approved by: [redacted] 8/9/13
Name, Senior Program Veterinarian Date

Approved by: [signature] 8/11/13
Name, Supervisor Date

UNIVERSITY OF WISCONSIN-MADISON
SCHOOL OF MEDICINE AND PUBLIC HEALTH
LABORATORY ANIMAL RESOURCES

STANDARD OPERATING PROCEDURE

NUMBER: 403a **EFFECTIVE DATE:** August 12th, 2013
TITLE: Sanitization of Cat Socialization and Behavioral Training Room

PURPOSE:	To outline procedures for the minimum sanitation and maintenance of cat socialization/behavioral training rooms
SCOPE:	All rooms designated as cat socialization/behavioral training rooms
RESPONSIBILITY:	Animal research technicians (ARTs) and supervisors working in designated areas
SAFETY:	PPE as required by room door signage
TRAINING REQUIREMENTS:	Self-study of this SOP and supporting documents

NOTE: This room is used by laboratory staff to provide dedicated, sanitizable space for cat socialization and behavioral training. Laboratory staff will notify LAR staff on days when room is utilized.

1. Sanitize equipment as follows:

Item	Frequency of Sanitization	Method of Sanitization
Floors	Daily (on days when used)	Mopping/spraying with approved disinfectant
Stainless steel racks/carts	Daily (on days when used) / Every 2 weeks	Wipe with approved disinfectant / cage wash
Mop heads/buckets	After each use/weekly	Dump and rinse after each use; cage wash weekly
Walls	Weekly	Mopping/spraying with approved disinfectant
Resting mats	Weekly	Washing machine
Sinks/tabletops/countertops	Weekly	Wipe with approved disinfectant
Feeders/water bowls/litter boxes (if present)	Weekly	Cage wash

Item	Frequency of Sanitization	Method of Sanitization
Room exhaust room filters	Weekly/as needed, or every 6 months.	Check/change-out
Enrichment items	Every 2 weeks	Cage wash
Functional floor drains	Every 2 weeks or as needed	Per area supervisor
Trash containers with liners	Every 3 months	Per area supervisor
Equipment containers and lids	As emptied, or minimum of every 6 months	Cage wash
Animal room doors/ door jams/ knobs	As needed or every 6 months	Per area supervisor
Exposed pipes/light fixtures	As needed	Per area supervisor

2. When applicable, label sanitized equipment with date of sanitization.

3. Record in applicable room log.

Name

Name
Attending Veterinarian, SMPH

Revision	Revision Description	Effective Date
0	Initial Release	August 12, 2013

Animal Transportation
Standard Operating Procedure

University of Wisconsin Madison
Yin Lab

SOP# 3 Date Revised: 7/10/13

Location(s):Room #Medical Science Building, 18 SMI, Animal housing area.
Responsibility: Yin Lab Staff
Safety: Personal Protective Equipment (PPE)

I. Purpose: To safely transport animals to and from the lab in preparation for daily lab activities.

II. Materials Needed:
Yin Lab Daily Animal Log
PPE
Dedicated animal kennel for each animal

III. Documents/Attachments:
Yin Lab Daily Animal Log

IV. Safety:
1. Authorization and a badge are needed to get to the 6th floor using the elevator.
2. Gown and booties are necessary to enter 6th floor.
3. Gowns and booties must be removed before leaving the restricted area.

V. Animal Transport Kennels
1. Each kennel will be assigned to a specific animal and labeled for use with that animal only.
2. The kennels are sanitized 1X/month by Laboratory Animal Resources (LAR) staff and this is documented in their sanitization log.

VI. **Directions**:
1. Documentation:
   a. Record animal weight on the Yin Lab Daily Animal Log
   b. Record weight on the upstairs food record.
2. Weigh the cat:
   a. Place the animal specific kennel on the scale and then zero the scale.
   b. Place the animal into the kennel and record weight.

3. Transport the animal in the animal kennel, covered with a drape, to the laboratory or play room. A cart may be used to transport the carrier.
4. After cleaning, experiments or feeding in the lab, or time in play room, return the animal to the animal housing area in the animal specific kennel with cover.

Approved by: Name 8/9/13

Name, Senior Program Veterinarian Date

Approved by: Jon C. J. 8/13/13

Name, Supervisor Date

Animal Equipment Cleaning
Standard Operating Procedure

University of Wisconsin Madison
Yin Lab

SOP# 7 Date Revised: 7/10/13

Location(s) Room # Medical Science Building, 18 SMI.
Responsibility: Yin Lab Staff

I. Purpose: To maintain a clean environment for the animals and help prevent infection.

II. Materials Needed:
Cetylcide II
Alcohol
Laundry detergent
Benchkote absorbent protector paper

III. Documents/Attachments:

IV. Safety:

V. Directions:

A. Each cat has a dedicated restraint bag, carrying kennel, and feed tube holder. Restraint bags and kennel covers will be laundered by lab staff 1X/3months. Kennels will be sanitized in the sanitizing washer 1X/ month by Laboratory Animal Resources (LAR). This sanitization will be documented and the cleanliness of the kennels will be monitored by LAR per their protocol. Feed tube holders will be washed with dish soap and rinsed with hot water after each use.

B. Shared items including the training platform, fixed head holder, cleaning table, and food tube tip will be sanitized between each use with cetylcide II for surfaces and alcohol for head holder and food tube tip. White Benchkote absorbent protector paper that covers the procedure table will be changed and dated 1X/3months.

Approved by: Name — 8/9/13
Name, Senior Program Veterinarian Date

Approved by: Jon C. J. Yin — 8/13/13
Name, Supervisor Date

Explant Maintenance
Standard Operating Procedure

University of Wisconsin Madison

Yin Lab

SOP# 1 Date Revised: 7/10/13

Location(s): Room # Medical Science Building
Responsibility: Yin Lab Staff
Safety: Personal Protective Equipment (PPE)

I. Purpose: To maintain chronic cranial implants for neurophysiological recordings, and maintain explant margins.

II. Materials Needed:

0.9% sterile saline solution (1liter bottle)
Sterile cotton swabs
Clean cotton swab
Sterile gauze
Non-sterile gauze
Variety of syringes (1cc–330cc)
Variety of needles (16g–23g)
Clean Kerr dental wax
Allen wrench
Alcohol swabs or bottled alcohol applied with sterile gauze
Suction
Yin Lab Daily Animal Log
Cleaning Solutions:
- Chlorhexidine (Nolvasan Solution: DNS) with sterile saline [2.5% of the 2% Chlorhexidine (.05% final concentration)]
- Povidine Iodine (Betadine Solution: BET) with sterile saline (150ml bag) [2% of the 10% Betadine solution (.02% final concentration)]
- Hydrogen peroxide (3% solution)
- Vetricyn
- Sterile saline (0.9% NaCl) (150ml bags)

Sterile ointments to be used on explant margins when necessary:
- TAO (Triple Antibiotic Ointment [Neo-Polycin])
- Other antibiotics, per RARC vets
- Cleaning instruments (stored in cold sterilization tray (Cetylcide G)
- 5 ¾ Glass Suction pipettes

Wax removal tool
Scissors (one for each cat)
Forceps (Three for each cat requiring recording cylinder cleaning)

III. Documents/Attachments:
Yin Lab Daily Animal Log

IV. Safety:
1. All personnel handling these specified animals must be adequately trained in methods of aseptic technique.
2. PPE including clean gloves are worn with all cleaning and applies to each section of this SOP. Wash hands prior to and after donning or doffing gloves.
3. A face mask must be worn at all times when cleaning recording chambers to minimize infection and protect personnel.
4. Put the animal in a restraint bag and, if needed, secure the head post.

V. Directions:

A. Documentation

1. Document appropriate cleaning procedures completed on the Yin Lab Daily Animal Log.

B. Recording Chamber Maintenance

1. Clean recording chamber every day recordings are performed or at least 2x/week.
2. Remove old wax if present with a clean wax removal tool. Wipe the outside of the cap and the chamber with alcohol swab or 2% betadine solution. Allow a minimum contact time of 2-3 minutes before wiping the surface clean using a new dry gauze sponge.
3. Loosen the side screws of the chamber cap with a sanitized (using alcohol swab) Allen wrench and remove the chamber cap.
4. Once removed, scrub the inside of the cap thoroughly with hydrogen peroxide or 2% betadine to remove any organic matter. Then soak the cap in 2% betadine solution for 10-15 minutes. Use a clean beaker that has been rinsed thoroughly with sterile saline.
5. Prior to cleaning the inside of the chamber, note the condition of the inside of the cylinder. Document any observations in lab notebook or log. Contact a veterinarian immediately if there are any concerns or if there is uncontrollable hemorrhage.
6. Remove glass pipette from cetylcide G and flush with sterile saline. Without touching the tip to ANY surface, attach the glass pipet to vacuum line, place base of the pipette onto weighted pipette holder and turn on central vacuum at valve.
7. Swab the injection port of the dilute betadine bag with alcohol. Pre-fill 25-mL syringe(s) with the dilute 2% betadine solution using a large-gauge sterile needle. Clean outside of cylinder top to bottom with

sterile q-tips soaked in hydrogen peroxide, then inside walls of cylinder bottom to top with new sterile q-tips soaked in hydrogen peroxide.

8. Flush the cylinder with hydrogen peroxide. Let soak for 1 to 2 minutes and remove fluid with pipette vacuum. Flush and fill the cylinder with the dilute betadine solution 2%, soak for approximately 1 to 2 minutes, and remove fluid with pipette vacuum. Continue the flushing process until the syringe is empty and the cylinder is clean. If necessary, repeat process with additional 25mL dilute betadine.
9. Swab the injection port of the sterile saline bag with alcohol. Pre-fill 25-mL syringe(s) with sterile saline solution using a large-gauge sterile needle. Flush and fill the recording chamber with sterile saline and remove fluid with pipette vacuum. Continue the flushing process until the syringe is empty and the cylinder is clean.
10. If there is granulation tissue at the base of the cylinder, use a sterile cotton-tip swab inside the chamber during the cleaning process to help remove debris. Do not apply too much pressure to the dura with the cotton-tip swab. Once a swab is used in the cylinder, do not use it again.
11. Chambers may or may not be packed with gauze between cleaning depending on how much fluid collects in the chamber between cleaning. Open gauze pack, then using sterile forceps and packaging (being careful not to touch gauze with fingers), fold the gauze in half twice to make it roughly the size of the chamber opening. This will avoid it touching anything exterior when putting it into the cylinder. Place sterile gauze in the chamber and leave it in until the next cleaning.
12. Rinse the cap (that has been soaking in betadine [#4]) with sterile saline or wipe with alcohol and dry it with sterile gauze. Place the cap back onto the chamber. Tighten the screws finger-tight; use caution not to over tighten.
13. Dispose of glass pipette in a sharps container.
14. Be sure to replace all cylinder caps prior to proceeding with cleaning of the skin-explant interface
15. Seal the gap with wax (kept clean in a closed sterile container) by using iron cautery, if necessary.
16. Additional Chamber Cleaning Notes:
    a. Do not touch the skin-explant interface with hands during cylinder cleaning. Never use betadine scrub, alcohol, agents containing alcohol, or agents containing chlorhexidine (e.g. nolvasan) inside the cylinders.
    b. Cetylcide G solution is prepared (per manufacturers directions) new every 30 days. The instrument dish filled with cetylcide G, used to hold sterile instruments is cleaned every 30 days. All instruments are soaked in cetylcide G for at least 24 hours and rinsed in sterile saline prior to use.

c. Once a sterile forceps touches anything that is not sterile (example, inside of the recording cylinder) it must be cleaned and sterilized before using it again. If the tip of the pipette is contaminated by any surface (hand, skin, counter surface) a new sterile (rinsed) pipette must be used.
d. Syringes labeled with animal identification, solution used, and date may be stored in a clean, dry drawer and used for one week.

C. Skin-explant Interface Maintenance

1. General:
   a. Clean gauze and instruments are used to clean skin-explant margins.
   b. Never use betadine scrub, alcohol, hydrogen peroxide, or Dakin's solution to clean the skin explant interface.
   c. Never scrub the skin explant interface with a brush or gauze pad during cleaning as this predisposes the skin to development of infection.
   d. As needed, use a small scissors to remove hair around the skin explant interface. Trim hair as close to the skin as possible leaving a 1-cm margin around the explant. Use caution when trimming hair around eye or ear coil wires that may be present along skin-explant interface.
2. Prior to beginning the cleaning procedure, note the appearance of the skin explant interface and document it in lab notebook or log.
   a. Palpate around the margin to assess for any accumulation of discharge under the skin.
   b. Notify the veterinarian if there are any concerns about the appearance of the skin explant interface prior to proceeding with cleaning.
3. Remove any solid dry crusts gently with a clean cotton-tip swab.
   a. Use sterile saline or Vetericyn or betadine over any areas with significant scabbing and allow it to sit for five minutes to soften the scab and help kill bacteria.
   b. If using betadine, rinse with sterile saline.
   c. Use another clean gauze pad to gently remove loose scabs.
   d. For new implants, only large scabs need to be removed as removing all scabbed skin may delay healing process.
4. Gently dry extra discharge/fluid on the skin-explant interface with clean gauze.
5. If betadine was not used, spray skin-explant interface with Vetericyn, avoiding exposure of the eyes and ears, and lightly pat dry any excess solution with gauze.
6. When necessary or under the direction of a veterinarian, apply a topical ointment such as Nolvasan ointment, triple antibiotic ointment,

or Silver Sulfadiazine around the skin explant interface. Use of any ointment must be documented in lab notebook or log.

D. Prepare dilute 2% betadine solution

1. Attach a large-gauge sterile needle to a sterile syringe. Draw 3.5 mL of 10% providone-iodine solution into the syringe.
2. Swab the injection port of the bag containing the sterile saline with alcohol and inject the betadine into a 150 mL bag of 0.9% NaCl to create a dilute betadine solution. Mix solution in bag.
3. Label the bag with preparer's initials and concentration of solution and date.
4. The bag of solution can be stored at room temperature for up to 10 days.

Approved by: [redacted] 8/9/13

Name, Senior Program Veterinarian Date

Approved by: [redacted] 8/13/13

Name, Supervisor Date

Non-Surgical Procedures: Anesthesia Required
Standard Operating Procedure

University of Wisconsin Madison
Yin Lab

SOP# 5

Date Revised: 7/10/13

Location(s) Room # Medical Science Building
Responsibility: Yin Lab Staff
Safety: Personal Protective Equipment (PPE)

I. Purpose: To communicate and document all lab procedures requiring anesthesia: Auditory Brainstem Response [ABR], Head Related Transfer Function [HRTF], making ear molds.

II. Materials Needed:
Yin Lab Daily Animal Log
Anesthesia Log, top page
Anesthesia Log, extra pages
Medical record

III. Documents/Attachments:
Yin Lab Daily Animal Log
Anesthesia Log, top page
Anesthesia Log, extra pages

IV. Safety:
All personnel handling these specified animals must be adequately trained in methods of aseptic technique.
PPE including clean gloves are worn and applies to each section of this SOP.
Wash hands prior to donning and after doffing gloves.
Put the animal in a restraint bag.

V. **Directions:**

A. Inform the vet staff by e-mail or telephone that a non-surgical procedure in the lab is scheduled to occur. If the procedure is an HRTF measurement, arrange for vet support for anesthesia.

B. Fast the animal for at least 12 hours prior to the procedure. Put the fast sign on the animal cage and communicate the fasting by writing the appropriate information on the white board, located on the floor, a day before the procedure.

C. Document all procedures requiring anesthesia on the anesthesia log and extra log pages. Document in the medical record.
D. Anesthetize the cat per protocol M00212.
E. Monitor animal vital signs per protocol M00212.

Approved by: [redacted] 8/9/13
Name, Senior Program Veterinarian Date

Approved by: [redacted] 8/13/13
Name, Supervisor Date

Food Preparation and Equipment Maintenance Standard Operating Procedure

University of Wisconsin Madison
Yin Lab

SOP# 4 Date Revised: 7/10/13

Location(s) Room # Medical Science Building, 18 SMI.
Responsibility: Yin Lab Staff
Safety: Personal Protective Equipment (PPE)

I. Purpose: To safely prepare food and maintain feeding equipment used for experiments.

II. Materials Needed:
- Purina Friskies moist food
- Hills Science Diet Optimal Care dry cat food
- Clean tap water
- Food blender
- Funnel
- Glass food beaker
- Masterflex tubing
- Needle nose pliers
- Bunsen burner
- Dish soap
- Cleaning brushes
- Sanitization/tube replacement stickers
- Daily animal logs
- Cetylcide II
- Alcohol

III. Documents/Attachments:
- Yin Lab Daily Animal Log
- Weekend feeding instruction form
- Upstairs food record

IV. Directions:
- A. Food preparation
  1. Only non-expired food will be used.
  2. Moist food is stored in original containers. Dry food is stored in an air-tight barrel that is sanitized by LAR staff monthly. Sanitization sticker with barrel change date is placed on barrel.

3. Moist food, dry food, and tap water are combined in the blender to the desired consistency, thin enough to move through the pump without clogging yet thick enough to form a small bolus that doesn't drip.
4. Food is funneled into the food beaker and used the same day. Any food remaining at the end of the day will be discarded.

B. Equipment cleaning and sanitation.
1. All food preparation items are washed in hot soapy water and rinsed in hot water.
2. Between each use, the experimental platform and food-tube tip are sanitized by wiping them down with alcohol or cetylcide II and letting them air dry (contact time at least 10 minutes). The food tube tip will then be rinsed thoroughly with sterile saline. This is documented in the Yin Lab Daily Animal Log. Each animal has its own dedicated food tube holder.
3. At the end of each day, remaining food is discarded and the food beaker and feed tubing is rinsed in hot water. Feed tube holder is washed in soap and rinsed in hot water.
4. Each animal has its own dedicated restraint bag and experimental harness if using one.

C. To repair/replace the food tube
1. Feeding tubes will be repaired when they become too stiff to fit well in the feeding pump, or are cracked and leaking. They will be replaced at least 1X/6 months. The last "date tubing changed" is specified on the food pump.

D. Documentation
1. Lab staff must document the amount the animal consumed in the Yin Lab Daily Animal Log and document in the upstairs food record that the cats were fed.
2. On the weekends and days that LAR staff feeds the animals, fill out a Weekend feeding instruction form and place one on each animal's cage.
3. Animals are fed in the animal housing area or in the lab by the lab staff unless otherwise indicated.

Approved by: Name 8/9/13
Name, Senior Program Veterinarian Date

Approved by: 8/13/13
Name, Supervisor Date

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Wednesday, August 21, 2013 1:23 PM
To: Name
Subject: RE: UW-Madison Progress Report

Thanks very much for this comprehensive report Name It appears responsive and thorough. I am in the office until 3:15 EST so feel free to call any time before then. 301-594-2061.
Axel Wolff

-----Original Message-----
From: Name [mailto: Name @wisc.edu]
Sent: Wednesday, August 21, 2013 1:13 PM
To: Wolff, Axel (NIH/OD) [E]
Subject: UW-Madison Progress Report

Dr. Wolff,
Please find attached a progress report and related SOPs.
I can provide additional information if you have time this afternoon for a quick chat.
When would be a good time for me to call?

Thanks
Name

M00212-0-10-10
protocol number

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

ANIMAL CARE AND USE PROTOCOL REVIEW HISTORY

Committee: **SMPH**

PI: **Yin, Tom C t**

Protocol no.: **M00212-0-10-10**

Title: **Behavioral and Physiolgoical Studies of Sound Localization**

APPROVED
FEB 22 2011
UW-Madison IACUC

Approval date: **2/22/2011**

Expiration date: **2/22/2014**

AMENDMENT HISTORY

	Date received	Date approved
	10-13-11	12-5-11
VC	7-30-12	8-7-12
VC	8-14-12	8-17-12
	1.17.13	8.26.13

Date received	Date approved

Research Animal Resources Center
University of Wisconsin-Madison Room # Enzyme Institute 1710 University Avenue Madison, Wisconsin 53726-4087
Telephone # Fax: Telephone # E-mail: help@rarc.wisc.edu www.rarc.wisc.edu

3rd Revision

Revision 02/2011

RARC Use Only: ___-___ ___-___ ___ ___ ___ ___-___ ___ ___ ___ M00212.0.10.10

Received AUG 26 2013

Orig 1.17.13

1st Rev: 7.23.13

2nd Rev: 8.9.13

UNIVERSITY OF WISCONSIN - MADISON ANIMAL CARE AND USE PROTOCOL REVIEW FORM

Forms should be typed or in computer-printed format. PLEASE MINIMIZE formatting changes when preparing on computer.
PC & Macintosh word processing forms can be downloaded via the RARC homepage: http://rarc.wisc.edu/
Return completed forms to RARC Room **Enzyme Institute, 1710 University Ave., Madison, WI 53726).**
Preferred method of delivery: attachment to e-mail (call [redacted] **or** [redacted] **or e-mail address).**
Hard copy not required except for the page with PI signature, which must be sent or faxed Telephone #

INVESTIGATORS: Animal protocols are assigned for review to the Animal Care and Use Committee(s) that provides oversight of the facility or facilities where the animals assigned to this protocol will be housed.
Questions? Call Name (Telephone #), Name (Telephone #), *or* Name (Telephone #) *at RARC, or consult the "Guide to Completing the Animal Use Protocol" on the RARC website.*

Submission Deadlines by College or School:

- School of Medicine & Public Health: 4:00 pm the 15th of the month
- School of Veterinary Medicine: rolling deadline
- Graduate School: rolling deadline
- College of Agricultural and Life Sciences: 4:00 pm 1st of the month
- College of Letters and Science: 4:00 pm on the 1st of the month

RARC Office Use Only:

_X__ Survival Surgery	_X__ Restraint	Amendment Stamp/Approval
_X__ Nonsurvival Surgery	___ Paralytic Agents	
___ Rodent Surgery	_X__ Fluid/Food Restrictions	
___ Nonrodent Surgery		
_X__ Multiple Major Survival Surgery	___ Nonstandard Housing	
	___ Nonstandard Husbandry	
___ Critical Veterinary Care		
___ Class B Dog/Cat	___ Occupational Health & Safety	
	___ Biohazards	
___ Exercise Exemption	___ Radiation	
___ Enrichment Exemption		

NOTE: ALL PROTOCOLS ARE VALID FOR THREE (3) YEARS FROM DATE OF APPROVAL.

1. **Principal Investigator/Project Director**: Tom C.T. Yin
   Telephone Numbers: Office: Telephone # Lab: Telephone # Animal Emergency: Telephone #
   Home: Telephone # Fax: Telephone # E-mail Address: tcyin@wisc.edu

   Alternate for animal emergency or study-related action/communication with Authority to act in the Investigator's absence:
   Name of Alternate for animal emergency/study-related action: Name
   Alternate Office Phone: Telephone # Alternate Phone: [redacted] Alternate Email: Name @wisc.edu

   Alternate contact for clerical purposes only for this protocol:
   Name of Clerical Alternate:
   Clerical Alternate Office Phone: Clerical Alternate Phone/Email:

2. **University Department** (of PI): Neuroscience Office Address Room # MSC
   Unit & Division Number (UDDS): A 53 5400

3. **Type of submission** (underline appropriate category): NEW RENEWAL AMENDMENT
   If Renewal or Amendment, please give current protocol code (e.g. G00180): Code: M00212

4. **This protocol is for**: TEACHING or RESEARCH (Underline all that apply) BIOMEDICAL; BEHAVIORAL; OBSERVATIONAL; AGRICULTURAL; FIELD; OTHER (SPECIFY)

5. **Title of this animal protocol**: Behavioral and physiological studies of sound localization

6. **Classification of animal use** (will be completed by RARC administrative staff): 1 2 3 4 5

7. Underline the appropriate response to each question below:

a) Will ANY surgery be performed on any animals?	**YES**	**NO**	If yes, fill out questions 24-30.
b) Will you be working with wild-caught animals?	YES	**NO**	If yes, fill out questions 31-34.
c) Will you be using nonhuman primates?	YES	**NO**	If yes, fill out question 35.

8. **Procedure locations:** Will any procedures on live animals (e.g., blood collection, injections, euthanasia, scans, etc.) be conducted in labs or other facilities outside of housing area? Underline one: **YES NO**
If YES, enter information on the table below, using additional lines as necessary. "Precautions" refers to steps taken to prevent potential disease transfer upon return to normal housing.

NOTE: ***Any location where animals are kept for more than 12 hours is considered HOUSING and should be included in Question 10.***

(hit "tab" in bottom right cell to add additional row)

Procedure	Building/Room #	Length of stay (hrs)	Method of transport & precautions, if any
Behavioral training, physiological recordings, cleaning implant margins, minor procedures (e.g. eye coil removal)	Room #	≤6 hrs	Opaque cage
MRI for locating inferior colliculus	Room #	<4 hrs	Opaque cage and LAR transport
Behavioral training, cortical cooling	Room #	<5 hrs	Opaque cage
Tissue perfusion during euthanasia	Room #	<2 hrs	Deeply anesthetized animal transported to procedure room in opaque covering; procedure performed in fume hood in room

9. **Species, Numbers, and Sources of Animals**
NOTE: TOTAL NUMBERS ARE FOR THE ENTIRE THREE-YEAR LIFE OF THIS PROTOCOL.

a. Numbers of animals needed for experiments for 3 years: *(hit "tab" in bottom right cell to add additional row)*

Species of animal	Total for 3 years	Source of animals (e.g. commercial vendor, another UW-Madison protocol)
Cat	10	ass A deal through LAR Purchasing, other investigators, or other commercial sources

b. Will any dogs or cats be obtained from Class B dealers? (Underline one) **YES NO**

NOTE: *Use of animals from Class B dealers* ***requires*** *permission from the Animal Care and Use Committee.*

c. **To ensure the health of laboratory animals, the Investigator must consider the previous use of animals on other projects.** The investigator must take into consideration how previous nutritional manipulations, blood draws, drugs and materials administered, and other manipulations may have compromised the animals' fitness for the proposed study in this protocol, or how the proposed study may adversely impact animals given their health history and assignment to earlier projects. **Animals that have undergone a major operative procedure, permanent physiologic alteration, or substantial impairment on a previous protocol are not eligible for major operative procedures on subsequent protocols.**
Have any of the animals listed in Question 9(a) been part of any other protocols (include breeding animals obtained from other investigators)? Underline one: **YES NO**
If YES, briefly explain how you have determined that the previous use of these animals will not compromise the animal's health and the research proposed in this protocol.

Animals received from other investigators will be experimentally naive and prior use will not compromise the research or health of the animals. All transfers will be approved by an RARC veterinarian.

10. **Housing**: Building(s)/facilities—including procedure room(s)—where the animals will be housed for more than 12 hours.

Animal housing facility in

11. Explanation of Goals, Animal Use, and Choice of Species

a. In straight-forward, nonmedical, nontechnical language that would be understandable to a layperson (aim for a high school-senior reading level), outline the specific scientific goal(s) and significance of this research. Be convincing as to why this work is important for advancement of knowledge, improving human or animal health, or for the good of society. Spell out all acronyms at first occurrence. **If this is a Renewal submission** please provide a brief (2-3 sentences) description of your progress and productivity in the past three years to help the Committee evaluate animal usage. This description can be a citation(s) to a publication generated from this research or new directions that will be pursued in the next three years. If a published manuscript is not yet available, a brief description of any other progress can be provided, such as abstracts, oral presentations, or presentations at meetings.

The overall aim of this project is to better understand the neural mechanisms of binaural ("two ears") interaction, or how input from the two ears – particularly in relation to sound localization – is integrated by the brain. Study results will help us understand how the brain integrates auditory information from the two ears. The neural circuitry involved in binaural interaction plays a major role in our ability to detect specific sound signals in the presence of background noise; loss of this ability is the major symptom observed in elderly people with hearing loss. A better understanding of the neural mechanisms involved in hearing processes such as binaural interaction can help in the design of hearing aids and in the development of hearing-therapy for humans. In recent years we have also discovered a new reflex that involves movements of the pinnae (external ears) of the cat, that is in principle the same as the well-known [redacted] observable in humans, where [redacted] during head movement. The reflex we discovered [redacted] We call this reflex [redacted] Gaining greater understanding of this reflex can help in increasing overall knowledge of important sensory-associated reflexes in humans.

Publications resulting from the work done in the past 3 years are listed below.

Tollin, D.J., McClaine, E.M. and Yin, T.C.T. Short-latency, goal-directed movements of the pinnae to sounds that produce auditory spatial illusions. *J. Neurophysiol.* 103: 446-457, 2010. PMCID: PMC2807232

Karino, S., Smith, P.H., Yin, T.C.T. and Joris, P.X. Axonal branching patterns as sources of delay in the mammalian auditory brainstem: a re-examination. *J. Neurosci.* 31: 3016-3031, 2011.

Ruhland, J.L., Tollin, D.J. and Yin, T.C.T. Gaze shifts of the cat to auditory and visual stimuli. *J. Assoc. for Research Otolaryngol.* (in press).

Tollin, D.J., Ruhland, J.L., and Yin, T.C.T. The role of spectral composition of sounds on the localization of sounds by cats. *J. Neurophysiol.* 109:1658-1668, 2013. PMC3602938

Gai, Y., Ruhland, J.L., Yin, T.C.T., and Tollin, D.J. Behavioral and modeling studies of sound localization in cats: effects of stimulus level and duration. *J. Neurophysiol.* 110: 607-620, 2013.

[redacted] unpublished

b. Specifically justify the use of animals for this research. Explain why it is imperative to use animals and why ***non-animal alternatives*** such as computer simulation or in vitro systems are not possible

At present there is no non-animal alternative that accurately models the complex neural circuitry and interactions involved in the sense of hearing. We have considered alternatives to the use of live animals in our studies, and in fact one of our goals is to produce a computer model of the neural circuitry involved in hearing. Models do help us to refine our questions, which in turn reduces the number of animals needed. However, in order to generate realistic computer models and to test their adequacy, additional physiologic data that can only be obtained from recordings made in animals are required. We simply do not understand the mechanisms of the brain well enough to simulate it with computer models or *in vitro* systems as of yet, therefore animal models are essential to achieving the scientific goals of this proposal.

c. Specifically justify why you chose the species cited in 9(a) for your work, such as the appropriateness of the species for your proposed work. Cost considerations are not justifications.

Cats are used for the following reasons: the physiological, anatomical and psychophysical characteristics of their auditory system are very similar to those of humans and higher primates. Their auditory system has been extensively studied by others such that most of the understanding of auditory physiology derives from studies in the cat, and the relevant parts of their brain are relatively easily accessible. The other animal species that have been extensively used in studies of sound localization are guinea pigs, gerbils, chinchillas, ferrets and barn owls. The rodents are not good models for studies of localization because the behavioral evidence indicates that their localization acuity is considerably less than that of predators like the cat, barn owl or human. For prey, they need only determine the general direction of a sound source, not its precise location. Barn owls are not good models since they are so highly specialized (they are the only

animals known to phase lock to frequencies above 3-4 kHz and they do not move their eyes) that results may not apply generally to other animals.

12. **Explain how the number of animals required was determined and justify that need.** Include all control animals and breeding colony animals in this discussion. A table may help clarify different experimental groups or studies and the specific numbers needed for each. Include any statistical analysis used (e.g. power calculations) in determining the animal numbers.

The number of cats needed for this study is determined by the scientific aims of the experiments and is governed by many different considerations. In these neurophysiological studies the number of neurons sampled, not the number of animals, is the primary criterion in determining the justification of numbers for this study. A large number of neurons need to be sampled in order to gain statistical viability. Based on past experience of the Principal Investigator (over 30 years at the UW-Madison), one experiment might yield only 10 successful recordings from neurons, while another might yield several hundred. The number of neurons required to reach statistical viability also depends upon the specific experiments being conducted and the differences recorded between individual neurons in any given experiment. For example, if all the neurons consistently show a strong effect, then relatively few neurons are needed to reach significance and therefore few animals are needed; but if there is considerable variability between the neurons then many more cells are required to demonstrate the presence or absence of an effect, e.g. in a t-test or an ANOVA with appropriate post-hoc analysis. While we have historically used about 2-3 animals/year for longer-term studies, this number is variable, depending upon the specific protocol-approved experiments that are being performed at any given moment. Ten animals over the three-year period of this protocol are requested. This number is derived from specific experience of the investigator, and is based on the average number of animals that the laboratory has used in the last 20 years. This number of animals represents the minimum required number of neuron recordings needed for sufficient statistical power to allow detection of biologically-relevant differences in neuronal and behavioral measures. In the event that this number of animals does not result in a sufficient number of neuron recordings to obtain sufficient statistical power, a protocol amendment to modify animal numbers will be submitted for ACUC review; the amendment will include pertinent recent statistics on neuron recordings to demonstrate to the ACUC the necessity for an increase in animal numbers. Not all of the animals are part of the neurophysiological experiments and some participate only in behavioral experiments. As with neurophysiological recording, there is considerable variability between animals in their ability to learn the challenging behavioral tasks, and the number of completed tasks is more relevant than actual number of animals. The number of animals requested in this protocol represents the minimum number necessary to collect data from behavioral trials that allow detection of statistically relevant results.

13. **Current or pending funding** for this project (add more entries as needed):

Title of Grant (1): Behavioral and physiological studies of sound localization
Funding Source (1): N.I.H. Grant Number (1): R01-DC07177

14. Identify the person(s) or animal care unit responsible for *daily* animal care:

Laboratory Animal Resources staff

15. Research/teaching staff expected to work with the animals in this study (*please delete examples*)

INVESTIGATORS: Everyone listed below must take the "Responsible Use and Care of Laboratory Animals" certification course before starting work with research animals. Protocols cannot be approved until PI and all listed personnel are certified. *RARC also offers several species-specific animal handling courses and procedures training (e.g. blood draw techniques, surgery). For information, call RARC* Telephone #

(hit "tab" in bottom right cell to add additional row)

Name / Degree / Phone number	Will work with the following species within this protocol	List the year each individual began working with the specie(s) and performing the procedures they will work with/perform in this protocol. **NOTE:** *For personnel who have worked with the named species less than 1 year, indicate who will train and supervise them.*
Tom Yin/ Ph.D./Telephone #	Cat	Cat: surgery, recording since 1969, sterile surgery since 1974
Name / MA/elephone	Cat	Cat: surgery, training and husbandry since 2003
Name BS/elephone	Cat	Cat: sterile surgery and training since 2008
Name Ph.D./elephone	Cat	Cat: sterile surgery and training since 11/2010
WNPRC veterinary anesthetists	Cat	Cat: multiple years anesthesia experience

RARC veterinary staff	Cat	Cat: multiple years anesthesia experience
Name	Cat	Cephalic implant surgery and maintenance since 1997, consultant only

Name from is collaborating with us as a formal visiting scientist on the experiment studying cortical inactivation. He developed the technique . For at least the first few surgeries he will come to Madison to help implant the

16a. Search for Unnecessary Duplication and Alternatives to Potentially Painful / Distressful Procedures

16a 1. UNNECESSARY DUPLICATION

The Animal Welfare Act and USDA Animal Care Policy #12 require PIs to assure the Committee that you have considered whether or not your proposed work unnecessarily duplicates existing knowledge. The USDA believes that database searches remain the most effective and efficient method for demonstrating compliance with the requirement to consider unnecessary duplication of research. To satisfy this requirement provide the following information:

(hit "tab" in bottom right cell to add additional row)

Electronic databases searched	Years covered by search	Date (MM/DD/YY) of most recent search performed	Frequency with which searches are performed (e.g. monthly)	Keywords used for this search
Pub-Med	1990-2013	6/19/13	Semi-annually	Sound localization, interaural time, interaural intensity, spectral cues, binaural, cat

Please provide a short narrative below of findings from your search. If your research will duplicate existing knowledge please state why this duplication is imperative to the attainment of scientific goals of the protocol.

Narrative 1: The literature search indicated that the research proposed in this protocol is unique and will not duplicate existing knowledge. I routinely monitor a wide variety of sources for information in the literature related to the problems of interest. I regularly read the two journals that are most relevant to my field: Journal of Neurophysiology and Journal of Neuroscience and monitor Hearing Research, Journal of the Association for Research in Otolaryngology, Science, Journal of Physiology (London), Nature, Journal of Comparative Neurology and Trends in Neuroscience, through subscriptions and copies available on the internet. In addition I regularly use EndNote, an electronic database that accesses Pub-Med to monitor papers published in book chapters or other journals. I regularly attend national and international meetings in which the most recent findings in the field are reported, and I communicate with a world-wide network of auditory physiologists over e-mail to keep up with the latest developments.

16a 2. Alternatives to procedures that may cause MORE THAN MOMENTARY OR SLIGHT PAIN OR DISTRESS

There may be alternatives to procedures that cause more than momentary pain or distress and that will not interfere with your research. Procedures that cause only momentary pain or distress are quick and minimally invasive, such as simple injections or blood collections, and typically do **not** include procedures performed under anesthesia. Do any procedures you have proposed cause more than momentary or slight pain or distress?

[ ] No
[X] Yes

If YES, USDA Animal Care Policy #12 requires PIs to assure the Committee that alternatives to procedures that cause more than momentary or slight pain or distress have been considered. To satisfy this requirement, the USDA believes that database searches remain the most effective and efficient method for demonstrating compliance with the requirement to consider alternatives to more than momentary painful / distressful procedures. Note that alternatives that do not allow the attainment of scientific goals of the research are not considered to be viable alternatives.

Use the keywords 'refinement' and 'alternative' in conjunction with each procedure that causes more than momentary or slight pain or distress and species. Note that pain management for each of these procedures should be addressed in Questions 18 and/or 27a and/or 29.

(hit "tab" in bottom right cell to add additional row)

Electronic databases searched	Years covered by search	Date (MM/DD/YY) of most recent search performed	Frequency with which searches are performed (e.g. monthly)	Keywords used for this search (e.g. "procedure + species + refinement+ alternative")
Pub-Med	1990-2013	6/19/13	Semi-Annually	Oculomotor, head restraint, ear muscle denervation, sound localization behavior, cat, feline, eye (or scleral search) coil, alternative, refinement, head post, cranial implant; food regulation; craniotomy; ear coils, thoracotomy, intracardiac perfusion; cortical cooling

Please provide a short narrative below of findings from your search. If an alternative or refined method was found, but cannot be used in your research, explain why this is the case.

Narrative 2: Literature search for alternatives to procedures that could cause more than momentary pain or distress did not reveal any scientifically acceptable alternatives to the procedures that are described in this protocol. For example, surgery is required to implant scleral search coils, and the coils may need to be replaced because of breakage after many months of use. Another method that could potentially be considered relies on visual reflective techniques, but this method is not adaptable to situations where the head is not restrained. Electro-oculograms, another method that could potentially be used in place of search coils, still require a surgical procedure and have not been used in our protocol due to their inferior accuracy regarding the study measurements we require. We will, however, adopt alternatives to procedures described in the protocol if any are found in the future that do not compromise the goals of the research.

For further guidance on conducting searches visit:
http://awic.nal.usda.gov/nal_display/index.php?info_center=3&tax_level=1&tax_subject=184
http://researchguides.library.wisc.edu/animalalternatives

16b. Occupational Health and Safety Considerations

Radiation or biohazard material usage in animals: In the table below, mark YES or NO for each category as it applies to this protocol. If YES, indicate the specific materials in the right-hand column and show the status (approved or pending) of Biological Safety (OBS-2) and/or Radiation Safety (99A) protocols.

Category	Used in project? (Yes/No)	If YES, list specific materials used
Recombinant DNA	No	
Genetically altered materials	No	
Infectious agents:	No	
Bacteria	No	
Virus	No	
Prion	No	
Other	No	
Carcinogen or mutagen	No	
Toxic agent	No	
Human-derived materials	No	
Teratogens	No	
Other		Formalin
Radioactive material	No	

Status of OBS-2 *needed for this project*: (Underline below OR check here): [X] Not applicable to this project.
PENDING APPROVED Provide OBS-2 number if approved:

Status of 99-A *needed for this project*: (Underline below OR check here): [X] Not applicable to this project..
PENDING APPROVED Provide 99-A number if approved:

c. **Special Precautions for Personnel:** If you are using any agent that could be hazardous to humans or animals, please provide any special precautions that should be followed by your lab personnel, animal caretakers, veterinarians, maintenance and/or sanitation personnel, or anyone else entering the areas where experiments are conducted or animals are housed. Include any special practices required for handling of any animal or experimental waste, animal carcasses, and cages and caging materials. Consider such requirements as masks or respirators, eye protection, lab coats, gloves, and disposal methods. Also consider posting signage for special requirements on animal room doors and/or cages.

Lab coats or dedicated uniforms are needed to enter the cat holding rooms. All personnel will wear a mask and exam gloves when working with cats with cranial implants to prevent possible inadvertent nosocomial infection of implant margins.

The only hazardous material that we use in the lab is 10% formalin on the rare occasions when an animal is perfused under anesthesia as a terminal procedure for tissue collection. Perfusions are done under a fume hood with gloves, eye protection and lab coats.

You must address Question 17 separately for each species.

17. **Description of Proposed Experimental Design/Studies**

a. In this section describe the animals' roles in your experiments—that is, the treatments and procedures the animals will receive outside of normal husbandry, from the first experimental manipulation to the final outcome. This response should provide the Animal Care and Use Committee with a clear understanding of what specifically happens sequentially to each animal or group of animals, and over what time period the procedures occur, including but not limited to:

- definitions of all materials given to animals, including dosage range, routes, and frequency of administration;
- blood draw methods, sites, and % volume
- breeding procedures/methods, if this protocol is to cover an animal colony or herd;
- the expected sequence, frequency, and duration of procedures;
- brief description of any devices/implants animals will receive, surgical and nonsurgical;
- the timing of any surgery within the experiment (do not repeat the surgical description you will provide in Question 28a);
- method, frequency, volumes, and numbers of biological samples taken;
- experimental diets;
- use of toxic agents, biohazardous materials, or radioactive materials (list in Question 16b);
- social or environmental manipulation;
- methods of antibody production.

Acclimation:

Since the procedures depend critically on the cats behaving in specific ways, cats are first acclimated to the laboratory setting by bringing them to the laboratory from the housing area and feeding them there so they learn that food will be provided in the laboratory setting. Initially cats are simply given a bowl of food, but gradually they are fed when they are inside a commercially-available cat-restraint bag so they become accustomed to eating while resting in the bag. Food reward is an effective, powerful and commonly-utilized motivator for behavioral training. To be successful, food is regulated and animal body weight is carefully monitored daily to ensure that animal health is not compromised during food regulation and food reward training. Each cat's working body weight will be established in consultation with veterinarians after an initial period of acclimation to the lab. Usually this entails a few weeks in which the cat's access to food is regulated to the period when the cat is brought to the lab. The cat will be provided with food *ad lib* during this period. The amount of food that each cat eats will be monitored to determine how much it needs to maintain a given weight. This process of determining a healthy working weight also provides an opportunity for the cat to acclimate to handling and being put into the cat bag. During this period no other procedures are performed except monitoring of the body weight. At the end of this acclimation period, in consultation with RARC veterinarians, the 'action weight' is determined, which is 85% of the working weight. Cats will be maintained at or above the action weight (see Q17c and Q19 for more information).

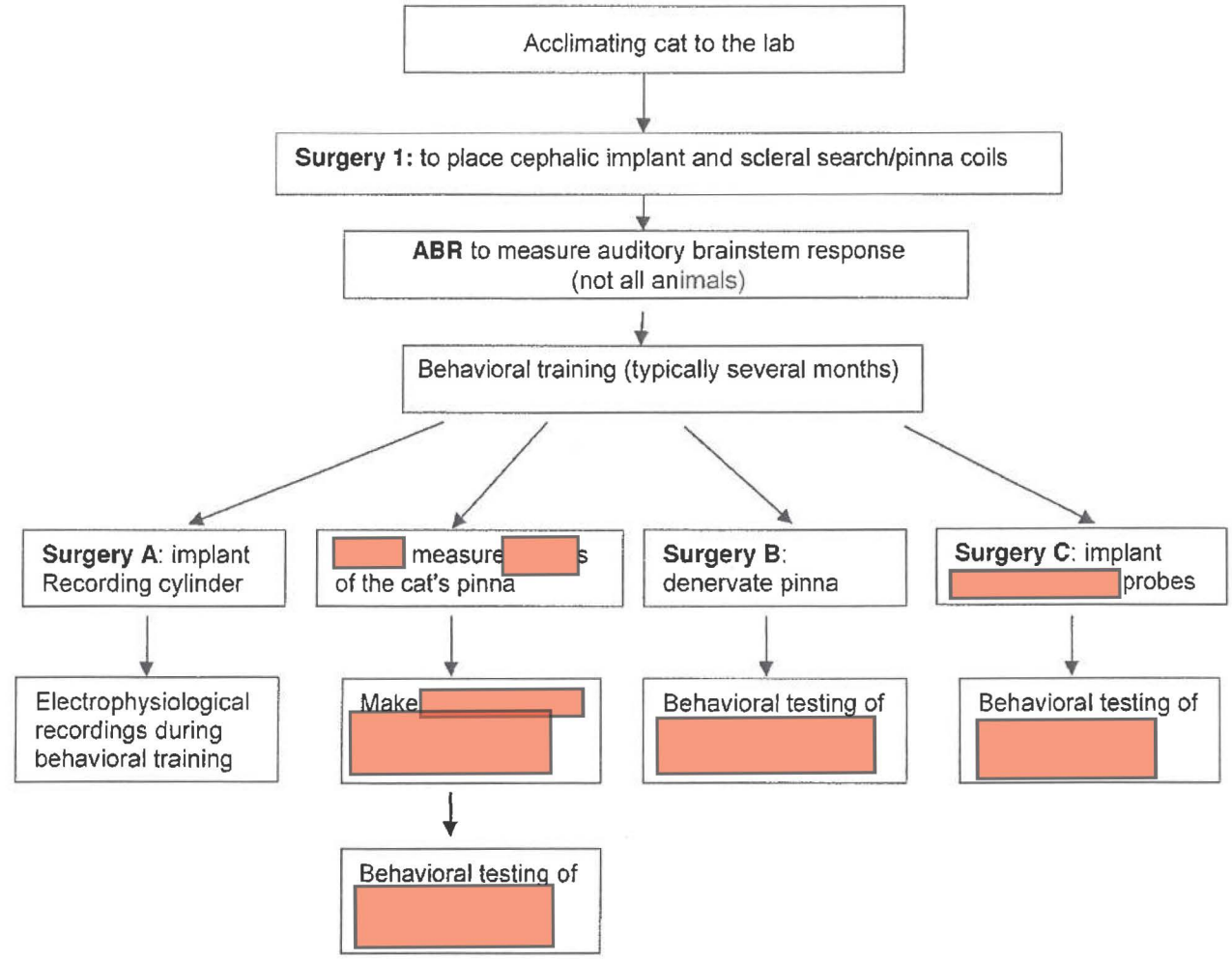
Instrumentation:

After the cats are sufficiently acclimated to handling and restraint, they will be assigned to one of several different experimental procedures. To minimize the number of surgeries that an animal undergoes, multiple surgical procedures

are combined when possible. However, multiple sequential surgeries are typically necessary because we need to allow for behavioral training between different surgeries and experimental procedures. The training regimen usually takes many months and the recording phase also extends over many months. If a cat has been previously trained or does not need to be trained, then the cephalic implant and scleral and pinnal search coils (Surgery1 - see chart below) may be combined with one of the other surgeries in one procedure. Cats that fail to adapt to the training regimen will be removed from the study.

Flowchart of experimental procedures

Note that no individual cat is assigned to all the procedures listed in this protocol, but will be assigned to a specific sub-set of experiments as indicated in the flowchart. In general, cats that are assigned to Surgery A and electrophysiological recording will not be used for [redacted] Surgery B and Surgery C. Cats assigned to [redacted], and Sugery B and C will not be used for Surgery A and electrophysiological recordings. Also note that several procedures, e.g. ABR and [redacted] do not involve any surgery but do require that the animal be sedated or anesthetized. ABR (auditory brainstem response) is a procedure to test the integrity and sensitivity of the auditory system using non-invasive techniques, by recording the averaged response from an electrode on the skin to repeated acoustic stimuli. [redacted] refers to a procedure to determine the way in which the head and external ear [redacted]



Surgery 1: Cephalic implant, scleral search coils and pinna coils; procedures required prior to initiation of behavioral testing

The sterile surgery needed to place the cephalic implant, scleral search coils and pinna coils will allow monitoring and localization of eye and ear position during the behavioral training and testing. The scleral search coil is needed to accurately monitor the position of the eyes. Since we are interested in the ability of the cat to [redacted] we use the natural behavior of the cat to [redacted] as a way of measuring their ability to [redacted] The use of a

coil for measuring eye movements is standard for neurophysiological studies of the oculomotor system. It has significant advantages over the electrooculogram in accuracy, stability and resistance to experimental artifact. In experiments described in this protocol, [redacted] are utilized to [redacted] in response to sound stimuli as a measure of where the cat perceives the sound [redacted]. Anesthesia and surgical details and drugs administered are described in Q#27a and Q28a and b. Typically [redacted] are implanted bilaterally. [redacted] are also [redacted] to allow monitoring of [redacted]. A coil placed in the [redacted] will be used to to monitor head position. As indicated above, if the cat has been previously trained or if training is not needed, then Surgery 1 may be combined with one of the other surgeries, such as the craniotomy and recording cylinder chamber implant of Surgery A.

ABR: to measure the auditory brainstem response (ABR) in order to determine hearing sensitivity.

For some of our experiments it is important to quantify the hearing sensitivity of the animal, as it is crucial for us to know that the animals can hear. Animals are sedated as described in Q27a, under ABR. The ABR is a standard procedure commonly used in human infants shortly after birth. It provides a relatively non-invasive measure of the sensitivity of an ear to sounds by recording and averaging the small electrical potentials generated by the auditory system to many hundreds of repetitions of a click stimulus. It is used to test the integrity of hearing and to track hearing thresholds. To record the ABR, fine needle electrodes are inserted aseptically in the subcutaneous tissue of the neck, ear and torso. The skin of the neck, back (between the scapulae) and pertinent areas of the torso are shaved to provide better electrode access. Three sterile #27 gauge needles will be inserted subcutaneously at the base of the neck, ear, and in the torso for recording purposes. Vital signs will be monitored every 15 minutes for the duration of the procedure, as is done for all other minor procedures in the lab. The animal will be kept on a heated surface to maintain body temperature. This procedure typically takes about an hour.

Behavioral training

Following recovery from the initial surgery (Surgery1) the cats are trained on a behavioral task for a period of several weeks to months. The cats are trained by having them work for a food reward in the laboratory. The cats will be trained under computer control to [redacted]. Initially the acoustic stimulus will be coupled with [redacted] The [redacted] Upon completion of a successful trial the cat will be given a food reward, a small amount of cat food in the form of a soft paste. Each day the cat will be expected to make [redacted] to a sound stimulus, but the number of successful trials varies considerably depending upon the difficulty of the task, the degree of training of the animal, and our goals in any given experiment.

Cats' weight and food consumption during the training sessions are monitored and recorded daily during weekdays. If the cat's weight falls below the action weight (determined as described under "Acclimation" above), RARC veterinarians will be consulted and food regulation/restriction will be modified or suspended until the weight surpasses the action weight; cats may be removed from all study activity during this time period if needed. In the last 14 years, there have not been any cases in which the action weight of an animal was reached. Training sessions last 1-4 hours per day for 5 or 6 days/week. Cats are allowed an average of 75 gm/day (range of 60-120/day depending upon the cat), or ~125 cc/day (range of 70-200 cc/day) of complete and balanced cat food on both training and non-training days and over weekends. It is important to emphasize that in order for this project to succeed, the cats must be motivated and eager to perform. Thus, concerted efforts are made to ensure that the cats are comfortable and content.

MRI Procedure

For purposes of recording [redacted] some cats may have an MRI prior to surgery to determine if the [redacted] is accessible to recording instrumentation, or if it is covered by [redacted] Cats will be transported to the WIMR facility for the MRI scan by animal care staff of Laboratory Animal Resources. The entire MRI procedure takes about 30 minutes. Animals are sedated as described in Q27a under MRI. If the MRI indicates that the [redacted] is covered by the [redacted] that animal will be used for another experiment. If the [redacted] is accessible to recording, the cat may undergo **Surgery A** at a later date.

Electrophysiologic recordings

After the cat has learned the specific behavioral task well, a craniotomy will be performed and an associated recording cylinder will be placed to allow electrophysiological recordings from neurons in the brain that are involved in [redacted] The recording cylinder is used to hold the microelectrode drive during daily recording sessions after the cat has recovered from the surgery. Anesthetic and surgical details are described in Q27 and Q28a. The electrophysiological recording is done by inserting sterile guide tubes to puncture the dura, followed by placement of fine

tipped microelectrodes into the brain through the lumens of the guide tubes. Since the dura has sensory receptors but the brain does not, puncturing the dura will cause a momentary pin prick sensation similar to getting an injection with a hypodermic needle, but passing the guide tube or electrode through the brain will cause no pain sensation. Awake human patients undergoing similar procedures report only a momentary pain sensation with no lasting effect. The guide tubes puncture the dura and protect the fine tip of the electrode from bending on the stiff dural surface. The recordings are then done on a daily basis over 2-4 months. Once passed through the dura, the electrode is advanced to the desired site. The brain itself is insensate so that once the electrode is through the dura, advancing the electrode in the brain will not cause discomfort. Electrodes are introduced using high-precision micro drives mounted to the recording cylinder. This approach allows for precise positioning of the electrode depth within a single session. Recent technological developments may allow a [redacted] At the end of an experimental session, the electrodes are removed, and the inside of the recording cylinder will be carefully cleaned and disinfected in a sterile manner, rinsed with sterile saline, and securely capped. When necessary, antibiotic solution or ointment will be applied in the cylinder.

Maintenance of implants

The margins of the implants are potential sites for infection and require maintenance to prevent infection. Maintenance can be performed with the cat in a restraint bag, and does not typically require sedation. This procedure usually takes less than 20 minutes and is performed at least once a week (or more frequently if needed). In consultation with RARC veterinarians, cleansers, ointments, and topical antimicrobials will be rotated to prevent selection of resistant microbes. Implant margins may be debrided, cleaned with hydrogen peroxide, betadine, chlorhexidine, Vetericyn, or other antimicrobial solutions as approved by RARC veterinarians, and rinsed with sterile water. Topical antibiotics may be applied as needed in consultation with a RARC veterinarian. The interior of the recording cylinder with a craniotomy will be cleaned and rinsed in a sterile fashion at least twice weekly. The cap will be removed and cleaned, the cylinder will be cleaned with 1-3% Betadine or other cleansing agents, rinsed with sterile saline, and the cap replaced. Antibiotic solutions may sometimes be infused into the recording cylinder to reduce the potential for infection, according to implant maintenance plans developed in conjunction with RARC veterinarians. When needed, and in consultation with the veterinary staff, culture and sensitivity testing of the inner recording cylinder or the implant margin will be performed to determine optimum therapeutic approaches.

Cleaning of dura mater

The combination of normal tissue re-growth and tissue stimulation caused by inserting the electrodes/guide-tubes through the dura will gradually cause the dura to harden. This ultimately prevents effective electrode insertion through the dura without causing damage to the fine tip of the microelectrode. As needed then, this hard granulation tissue will be removed under anesthesia (see Q27a for anesthesia details). The procedure typically involves using sterile technique and instruments to gently scrape-away the granulation tissue. The animal's head will be safely secured and stabilized during granulation tissue cleaning and removal. The procedure takes about an hour. Bacterial colonization of the tissue within existing recording cylinders is not uncommon in studies of this type, therefore several precautions will be taken to prevent local or systemic bacterial contamination during dural cleaning, including, pre-procedural administration of systemic IV antibiotics and aseptic cleansing of the cylinder and tissue before, during, and after cleaning, drilling or removal of tissue. If the dura is penetrated during the procedure, RARC veterinarians will be informed and an appropriate management plan will be initiated. Despite all precautions, there is a possibility that systemic infection may occur. RARC veterinarians will be informed of laboratory activity at all stages, particularly if infections are suspected.

After the dura hardens or if the skull bone regrows to fill the craniotomy, cleaning/scraping of the dura may not be sufficient, and a repeat craniotomy may be required (as described in Q28a) to allow for continued neural recording.

[redacted]

In some experiments [redacted] will be measured to determine the acoustic input to the ears [redacted]. The [redacted] that describe for each ear [redacted] It allows us to produce a [redacted] when stimulating the ears [redacted] i.e. the subject will [redacted] In this way we can stimulate the cat [redacted] but it will respond as if t[redacted] By modifying the [redacted] we can test the hypothesis that t[redacted] Cats are sedated or anesthetized as described in Q27a. Note that there is no surgery in these procedures, but animals must be sedated so they are immobile during the acoustic measurements.

[redacted]): To implement a [redacted] in the behaving cats, the cats hearing will be stimulated via [redacted] The [redacted]

[redacted] We are following the general strategy described by [redacted], but adapting the technique to cats. The [redacted] and we will use this [redacted] to study the [redacted]

To perform the [redacted], individual cats will be made, as there is considerable variability in the [redacted] of each cat. To make the [redacted] which will be done under light anesthesia (see Q27a). [redacted]. It is anticipated that the cats will initially require some period of adaptation to wearing the [redacted]. Cats will wear it for short times initially, and exposure time will gradually increase. If the cats effectively wear the [redacted] during shorter initial experiments, they may wear them for longer periods during the day to further acclimate them to wearing the [redacted]. [redacted] will be inspected periodically for any deterioration or breakage, and repaired as necessary. In addition the [redacted] will also be visually inspected to monitor irritation caused by the wearing [redacted] will be used to determine 1) how [redacted], 2) if cats [redacted] defined by the [redacted] and 3) t[redacted]

Surgery B: to immobilize the [redacted]

To study the effects of [redacted], the [redacted] in animals that are well-trained. See Q27 and Q28a for details on anesthesia and the surgical procedure. It is hypothesized that the cats will show [redacted]. The [redacted] The effectiveness of the [redacted] can be easily assessed by the measurements of the [redacted] after the cat has recovered from the surgery.

Surgery C: to implant [redacted]

To test the hypothesis that [redacted], we propose to use [redacted] while testing the cat's [redacted]. To [redacted] This method, as compared to others such as [redacted] has the advantage of being rapidly reversible. The approach to this surgical procedure is the same as that described for other sterile surgeries except that a craniotomy is necessary to place the [redacted] (see Q#28a for details). All implanted items are sterilized either by autoclaving or by gas sterilization. The [redacted] is anchored to the surrounding bone with dental acrylic. Dural margins are sutured closed as much as practical and absorbable Gelfilm is used as necessary to close the opening. Any gaps between bone and [redacted] are filled with absorbable Gelfoam to prevent the overlying dental acrylic from contacting the brain. This technique has been developed by Name ([redacted]. Name who is a Professor of Title will be assisting the initial surgical procedures for implantation of [redacted] as a visiting scientist. To ensure that the [redacted] was effective, the animals will be tested on their ability to [redacted], a task that Name lab has shown to be dependent upon [redacted] An RARC/SMPH veterinarian will be present to observe the procedure the first time it is performed, and a report of the procedure and a progress report of any experimentation will be provided to the SMPH ACUC at the next scheduled meeting. The procedure will not be performed on any additional animals until the ACUC has had the opportunity to review the outcomes of the first procedure.

During the experimental sessions of [redacted] each cat will undergo multiple [redacted] Each session consists of an initial period without [redacted]. Evidence in the [redacted] shows that the [redacted] take about 1-2 minutes. The effect of [redacted] We expect that animals will not be negatively affected by the [redacted] since the behavior and electrophysiological results from the [redacted] show complete recovery within minutes. Furthermore, anatomical examination of the brains of cats that have undergone [redacted] show no visible effect of the procedure. We will begin by allowing a day of recovery following a [redacted], but this may be shortened as outcomes are assessed. Each session will take about 2-3 hours.

Pharmaceutical-grade compounds will be used whenever possible. If non-pharmaceutical-grade compounds are used because of the unavailability of pharmaceutical-grade equivalents, compounds will be prepared, stored and used as described in the All –Campus Policy and SOP on the use of non-pharmaceutical grade materials (http://www.rarc.wisc.edu/policy/2010-037.html)

b. Do any animals undergo any type of restraint beyond normal housing methods? (examples of non-normal housing include metabolic crates and restraint chairs). Underline one: **YES** **NO**
If YES, describe the method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. If you are unsure whether or not your proposed methods are considered restraint, contact your Attending Veterinarian.

In some experiments, cats' heads are held in a fixed position by a clamp attached to the steel post that is a component of the [redacted] For all experiments within the acoustic sound chamber the cat is placed into a commercially-produced nylon cat-restraint bag to minimize movements during training and recording. The cat can freely move its limbs in the bag but cannot ambulate. The [redacted] and head restraint is needed for those experiments in which the head must be maintained in a known position, as the position of the head relative to the acoustic stimulus is the primary variable in the acoustic cues for sound localization. A typical training or recording session will last from 1.5 to 3 hours, after which the cats are allowed time to run free in a controlled environment supplied with enrichment items. Cats are never restrained for over 4 hours. If the animal shows any discomfort at any time during the training or recording period, or if there are any clinical signs or behaviors that indicate undue stress, the experiment will be immediately terminated for the day. Cats that do not successfully adapt to the restraint practices will be removed from any experiments that require the restraint.

c. Are any animals subjected to fluid or food restriction or regulation? Underline one: **YES** **NO**
If YES, discuss type and length of restriction, the expected consequences of restriction on the animals' health and well-being, and justification for such restrictions.

Access to food is regulated/restricted for all the animals in their home cages for the period when they are being trained and tested. Cats are fed only in the laboratory during the behavioral training and testing. This food regulation provides positive means of rewarding learned behaviors. This is necessary to motivate them to perform the required behaviors that allow attainment of scientific goals. As described above in Q#17a, the cats' weight and amount of food consumption during the training sessions are monitored daily to ensure that they are getting sufficient nutrition. Typically during daily training, the cats earn enough food rewards to satiate themselves. Training sessions last 1-4 hours for 5 or 6 days/week and the cats are allowed 75 gms/day (range of 60-120/day depending upon the cat) or ~175 cc/day (range of 140-280 cc/day) of food during training or on non-training days such as over the weekend. If the body weight falls below 85% of the established normal 'working weight', controlled-access feeding is suspended until the weight is regained. Weight is recorded for each training and recording session. We consult regularly with the RARC veterinarians regarding the food regulation or restriction of each animal, and ensure that weight does not fall below the 'action weight' of 85% of working weight. It is not uncommon for there to be a hiatus in the training or testing of any given cat for weeks or even months. During any such hiatus, cats are provided free access to an appropriate amount of food based on normal healthy weight. On non-training days, cats will be provided measured amounts of food in their home cage. Animals not undergoing behavioral training, during recovery from a surgical procedure, or removed from study for medical reasons may be provided additional or *ad lib* nutrition. Supplemental food in the home cage is typically not provided if an animal does not receive some minimal amount of food during training, as this would positively reinforce the non-performance of necessary behavioral tasks and thus affect study outcomes. However, if an animal's performance results in an inadequate amount of food reward being provided for more than 2 consecutive days, veterinary consultation will be sought in these rare cases.

d. Will any animals require nonstandard husbandry or housing exemption (e.g. exercise exemption, modified light cycle, extended cage cleaning periods, nonstandard cage type or size, etc.)? Underline one: **YES NO**
If YES, indicate the type of nonstandard husbandry required and scientific justification for these practices.

Instrumented animals are singly- housed in cages overnight when they are not under direct supervision to prevent implant damage from allogrooming because of the possibility of one cat licking or grooming another cat and dislodging an electrode or electrical connector. The animals are taken out of their home cages on a regular basis and allowed to roam freely and interact with laboratory and/or animal care personnel in a dedicated and sanitizable enriched environment within vivarium space. Cats usually receive hours of daily play and social time.

18. Will animals be subjected to **more than momentary or slight pain or discomfort** as a **result** of the experimental or other study-related procedures? Underline one: **YES NO**
If YES describe the analgesics you will provide. Include drug names (generic preferred), dosages, route of administration, nursing care, mechanical devices, etc.

All surgical procedures will be performed under general anesthesia. For procedures that may potentially be painful, opioids (e.g. buprenorphine) and/or Non-steroidal Anti-Inflammatories (NSAIDs) such as meloxicam or ketoprofen will be used. Buprenorphine is administered IM, Bucally, SC or IV at a dosage of 0.005-0.01 mg/kg, with initial dose provided during surgical procedures, then post-operative administration q6-12 hours for 3-5 days. Meloxicam regimen is 1st dose 0.1-0.2 mg/kg SC, then 0.05 mg/kg PO q24h for 3-5 days post-operatively. Ketoprofen regimen is 1-2 mg/kg SQ once daily for no more than 3 days post-operatively. Topical analgesics (Lidocaine ointment or cream) may be applied to the margins of some of the surgical sites. Administration of these or other appropriate analgesics and necessary regimens may be utilized at any time under direction of an RARC veterinarian. Clinical signs indicating analgesic treatment may be needed include (but are not limited to) inappetence, hunched or abnormal posture, inactivity, or abnormal vocalization.

NOTE: If all experimental or other study-related procedures are **terminal** and therefore performed only on anesthetized animals, type an X between the brackets: [ ]

19. Describe how frequently animals will be monitored to ensure they are not experiencing pain or discomfort from your procedures **or** any unanticipated illness or injury not necessarily directly related to your research. Describe the criteria or clinical signs (e.g. ruffed fur, hunched posture) that you will use to determine when euthanasia will be performed in these cases.

Laboratory members interact with the cats typically 5 days/week, and the animals' health and well-being are closely monitored. LAR personnel monitor the animals 7 days/week. In order for the experiments to succeed, it is essential that the animals are comfortable. Concerted efforts are therefore directed toward assuring that animals are not stressed. If there are any compromises to animal well-being (even minor), this is quickly reflected in their behavioral activity. Changes caused by any untoward impact on well-being are therefore easily detected during the daily monitoring of animals as the study progresses. It is emphasized to lab personnel that the single most important parameter in the behavioral training is the well-being of the animal.

Implant margins are routinely cleaned and disinfected at least weekly, and typically more often, with sterile water or a veterinary-recommended cleaning/disinfecting agent. Topical antibiotic ointment is applied if needed, as recommended by RARC veterinarians. If the eye coils have detached from the sclera, coils will be reattached or removed (see Q#17a above for details). Potential ocular or conjunctival irritation or infection can occur in these types of studies, as is inflammation or local infection around implant margins.

Veterinary consultation will be sought in the event that issues are noted, such as inflammation/infection of implant margins or tissue irritation from coils.

Bacterial colonization of the tissue within existing recording cylinders can occur, and precautions will be taken to prevent local or systemic bacterial contamination, including administration of systemic antibiotics and aseptic cleansing of the cylinder and tissue before, during, and after dural cleaning. If the dura is ever unintentionally penetrated, RARC veterinarians will be informed and an appropriate management plan will be initiated.

Despite all precautions, there is a possibility that systemic infection may occur. RARC veterinarians will be consulted at all stages if infections are suspected or noted.

If weight-loss greater than 15% of the working-weight of each animal is noted (i.e. weight falls below the action weight), RARC veterinarians will be informed, and an appropriate management plan will be implemented; animals will be humanely euthanized if there is not an acceptable response to treatment, as determined by veterinarians.

In the event of a loss of the [redacted] the animal will be immediately sedated or anesthetized (as described in Q27a), so that examination and treatment can take place. During examination and treatment, the integrity of the bone will be carefully evaluated in consultation with the RARC veterinarians. Treatment will be determined by RARC veterinarians, but typically consists of removal of any [redacted], and suturing of the skin to cover any exposed bone. Over time, X-rays of the skull may be taken to help fully evaluate bone integrity. If the integrity of the bone table is determined to be stable and healthy, RARC veterinarians can approve replacement of the [redacted] (performed as described in Q28a). If the integrity of the bone table has been compromised, then the cat will be humanely euthanized in a timely manner or transferred to a different study if medically and scientifically appropriate.

Possible complications of [redacted] include inadvertent blood vessel puncture. Since the penetrations are done through the dura, it is not possible to see the large vessels in order to avoid them. Signs of such damage include subdural hematomas, or other bleeding that would be visible through the dura, or neurologic abnormalities indicating lesions of the brain tissue. RARC veterinarians will be consulted for such situations, and veterinary orders will be followed, including possible humane euthanasia

The [redacted] also carries some degree of risk for infection, edema, or hemorrhage. A collaborator assisting with this protocol (including with initial surgeries) has over 20 years of experience with [redacted] and will serve as an important consultant for any problems. RARC veterinarians will be consulted if there is any indication of problems with the [redacted]

If there is an unanticipated illness or injury, as evidenced by clinical signs such as inappetence, lethargy, sub-optimal body condition or abnormal vocalization, RARC veterinarians will be contacted. Appropriate treatment will be initiated, and the animal will be given time off from working in the laboratory until there is veterinary approval for the animal to return to active study. If any problem is unable to be resolved, the animal may be euthanized under guidance or direction of an RARC veterinarian, or transferred to a different study if medically and scientifically appropriate.

Ultimately, the laboratory depends on the advice and consultation with RARC veterinarians to make decisions on whether animals that are in discomfort should be euthanized or not. The veterinarians have the full authority to initiate treatments, remove animals from study or humanely euthanize them as needed. These decisions are always made with the three R's of animal care (replacement, reduction and refinement) in mind. Over the last 20 years we have greatly reduced the number of animals used in our experiments, while maintaining animal health and well-being at the highest levels.

20. Describe the **specific criteria** for termination of animals **if experiments could induce chronic disease, tumors or radiation sickness.** These criteria should be described in terms of tumor size, specific animal characteristics or behaviors, weight loss changes, observed clinical signs, etc.

The care and management of issues that may be associated with long-term implants or other study-related activity, and criteria for euthanasia, are described in Q19.

> **NOTE**: *If experiments are not expected to induce these conditions, please type an X between the brackets*:
> [ ] **Chronic disease, tumors or radiation sickness are not anticipated**.

21. Describe the methods of euthanasia used, including drugs, dosage, and any sedation. Consult the 2007 *Report of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia (*www.avma.org/resources/ euthanasia.pdf*)* or your school's Attending Veterinarian for appropriate euthanasia methods. **Even if euthanasia of animals is not part of this project, complete this Question for cases of unanticipated illness or injury.**

> **NOTE:** *In general, physical methods (cervical dislocation, decapitation) are recommended for use only after other acceptable means have been excluded, in sedated or unconscious animals when practical, when scientifically or clinically justified, and with Animal Care and Use Committee approval. Physical methods* ***without*** *pre-anesthesia* ***require*** *scientific justification and description of the training of personnel who will perform it.*

All cats are euthanized by an IV overdose (100-150 mg/kg bw) of sodium pentobarbital or a pentobarbital euthanizing solution (for example, Beuthanasia®). Death is confirmed by the cessation of respiration followed by cessation of the heartbeat.

For animals in which brain recordings were performed, there is a scientific need to preserve the brain tissue in order to confirm the location of electrode placements. A terminal perfusion of 10% formalin is therefore performed at the time of euthanasia. The procedure for perfusion consists of initial anesthetic induction with ketamine hydrochloride (15-20 mg/kg) and acepromazine (.2 mg/kg) given intramuscularly. An overdose of sodium pentobarbital or other euthanizing solution is

then administered IV (100-150 mg/kg bw). Before the heart stops beating, but while the cat is under a deep plane of surgical anesthesia, the chest wall is opened and a cannula is inserted into the left ventricle of the heart to allow warmed saline (about 500 ml, or until the blood runs clear), followed by warmed 10% formalin (about 1000 ml), to perfuse the cardiovascular system.

22. If the animals are not euthanized at the end of the study, what will happen to them? Include descriptions of transfer of animals to other approved animal care and use protocols, or return of animals to managed colonies or herds.

Some animals may be transferred to other investigators with the approval of a veterinarian.

23. Could any animals or animal products involved in these studies possibly be consumed by humans? Underline one:
YES NO
If YES, list any drugs to be given to the animals and the recommended withdrawal times before safe consumption:

INVESTIGATOR SIGNATURE:
To the best of my knowledge, I certify that the information provided in this Animal Care and Use Protocol is complete and accurate. I understand that approval must be renewed annually, that every third year the ACUC must perform a new review of my protocol, and that I might be required to complete a newer version of the Animal Care and Use Protocol and provide additional information at the time of the triennial review.

I also understand that ACUC approval must be obtained by an amendment to this protocol before I:

- Use additional animal species, increase the number of animals used, or increase the number of procedures performed on individual animals;
- Change procedures in any way that might be considered a significant departure from the written protocol;
- Perform additional procedures not described in this Animal Care and Use Protocol;
- Allow other investigators to use these animals on other protocols, or use these animals on another of my ACUC-approved protocols.

I further certify that:

- No personnel will perform any animal procedures until they have been approved by the ACUC, via RARC. When new or additional personnel become involved in these studies, I will submit their qualifications, training, and experience to the ACUC and seek ACUC approval before they are involved in animal studies;
- I will ensure that all personnel are enrolled in an institutional Occupational Health and Safety Program prior to their contact with animals, or have declined in writing to participate, if allowed by local policy;
- I will provide my after-hours telephone numbers to the animal care staff in case of emergency.

I plan to follow the provisions for the care, use and treatment of animals found in the NIH "Guide for the Care and Use of Laboratory Animals," or the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching". I assure that these procedures do not unnecessarily duplicate previous experiments.

Signature of PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: _____

(A signature is required for submission. Either print, sign, and fax this page to Telephone # *with a cover sheet that identifies you/your protocol clearly, or paste an image of your handwritten signature here.)*

Questions for Projects Involving Surgical Procedures

24. Give the names of all research staff who will perform hands-on surgery on the animals in this study. For each person listed, describe their type and length of surgical training and experience, emphasizing **specific** experience with surgeries to be performed as a part of this study. For personnel listed below who have less than 1 year of experience with the surgeries they will be involved with, indicate who will train and supervise them. *Please delete the examples are provided in the table below for you.*

(hit "tab" in bottom right cell to add additional row)

Name/Phone Number	Brief description of SURGICAL training/experience.
Tom Yin / Telephone #	Acute Surgical procedures with cats surgery since 1969; sterile cat and monkey surgery since 1974; experienced with terminal perfusion of cats since 1970. All procedures.

Name / Telephone #	Assistance with surgical procedures and staff training since 2003. All procedures.
Name / elephone	Cat surgery since 1986. All procedures.
Name elephone	Surgical experience with cats since 2009. All procedures.
Name	Cat surgery experience since 2011. All procedures.
Name	Surgical consultant for some of our surgical procedures. Cranial implant and craniotomy surgeries since 1997.

Name from is collaborating with us as a formal visiting scientist and for the first few surgeries he will come to Madison to help implant the

25. Where will surgery be performed? Room number(s): Building:

Sterile surgeries in Room # ISC; minor procedures in Room # ISC o Room # Bardeen

26. How many animals listed in Question 9(a) will undergo surgery?

All

27. **Anesthetics and Paralytic Agents**

a. Describe anesthetic method used, including all drugs, dosages, routes of administration and supplementation regimen. Include how anesthesia level is monitored, e.g., list the physiologic parameters that will be monitored to ensure adequate anesthesia depth for both general and local anesthesia. *Documentation of the anesthesia used and the monitoring of anesthetic depth is* ***required*** *for all surgical procedures.*

Surgery 1 (head post, ear/ eye coils), Surgery A (recording cylinder placement), Surgery B (pinna denervation)

Cats will be premedicated with 0.1 ml (.03mg/ml) buprenorphine (0.005-0.01 mg/kg), IM, SQ, IV or sublingually, and the animal will be intubated following the initial anesthesia with ketamine hydrochloride (10-20 mg/kg) and either acepromazine (0.2 mg/kg) or dexmedetomedine (25 ug/kg), and placed on 100% oxygen and 0.5-3% isoflurane. Mechanical ventilation may be used. The animal will be kept warm throughout the procedure on a warm- water blanket. An IV catheter will be placed and IV fluids will be administered at 5ml/kg/hr for the first 2 hours, then 2.5ml/kg/hr for the duration of the surgery. If the surgery is extended, the volume may be reduced to prevent fluid overload. Eyes will be flushed with sterile saline every 15 minutes throughout surgery until an ophthalmic ointment is applied.

Depth of anesthesia is carefully monitored by the anesthetists using heart rate, respiration, CO_2, O_2 (e.g. via pulse oximetry) and any response to stimulus of the animal. Parameters such as heart rate and respiratory rate, as well as body temperature, will be recorded at least every 15 minutes until extubation. The animal will be kept warm and monitored carefully until sternal and returned to its cage.

Cefazolin (20 mg/kg, IM, IV or SQ), or other antibiotic as prescribed by an RARC veterinarian, may be administered at the time of surgery of surgery.

Surgery C: Implant

To implant the anesthesia is induced with ketamine and acepromazine as described above (e.g. for Surgery 1), and maintained either with isoflurane (0.5-3.0%) or pentobarbital. Pentobarbital anesthesia will be administered by IV Continuous Rate Infusion as developed and overseen by RARC veterinarians and/or other veterinary specialists, or by other methods as approved in advance by RARC veterinarians. If isoflurane is used, induction will be followed with intubation and oxygen with possible mechanical ventilation if necessary. Monitoring of anesthetic depth is as described above. The visiting scientist/collaborator on this project, who has been implanting for over 10 years, prefers pentobarbital rather than isoflurane because isoflurane is reported to cause increased intracranial pressure. Plumb's *Veterinary Drug Handbook*, 5th edition, 2005, cautions against use of isoflurane in cases where Cerebrospinal fluid (e.g. increased cranial pressure) may be a factor.

Cefazolin (20 mg/kg, IM, IV or SQ), or other antibiotic as prescribed by an RARC veterinarian, may be administered at the time of surgery.

Non-surgical procedures, lasting 1 to 2 hours

ABR, eye coil repair/removal, , MRI,

For these minor, non-invasive procedures requiring only sedation or lighter anesthesia, cats will be sedated using one of the following regimens: 1) Dexmeditomidine (up to 0.5 mg/kg IM), 2) Dexmedetomidine IM and ketamine (0.01-0.02, 2-10 mg/kg IM respectively), 3) Acepromazine (0.2 mg/kg) and Ketamine (10-20 mg/kg), 4) ketamine 10-30 mg/kg, or 5) a combination of ketamine (2-10 mg/kg), dexmedetomidine (0.01-0.02 mg/kg), butorphanol (0.2 mg/kg)or buprenorphine (0.01 mg/kg). Antisedan® (Atipamezole) (0.15 mg/kg IM or slow IV at 0.5 -1 times the volume of administered dexmedetomidine), may be administered as a reversal agent for dexmedetomidine during recovery.

For some procedures that may require an additional dose of ketamine, one-half to the full initial dose may be administered to maintain adequate sedation or anesthesia, particularly for sedation procedures lasting longer than 30 minutes. Repeat doses of 5 - 10 mg/kg ketamine can be repeated up to twice in a two hour period; veterinary approval will be sought before the administration of a higher dosage or more frequent ketamine dosing.

For some procedures lasting less than 2 hours, animals may also be given atropine methyl nitrate (0.15 mg/kg) or atropine sulphate (0.04 mg/kg) to reduce respiratory secretions, if approved by an RARC veterinarian.

Cats are anesthetized initially with either 1) ketamine (10-20 mg/kg) and acepromazine (.2 mg/kg) IM, or 2) a combination of ketamine 2-10 mg/kg, Dexmeditomine (0.01-0.02 mg/kg) +/- buprenorphine (0.01 mg/kg) or butorphanol (0.2 mg/kg) IM if pentobarbital is not utilized. Anesthesia is typically maintained with sodium pentobarbital IV diluted (50:50) with sterile saline and given as needed to maintain an appropriate level of anesthesia (usually about 10-20 mg/kg initially and 3-5 mg/kg/hr in the steady state). Pentobarbital has in most instances been superseded by inhalation anesthetics, but the acoustic demands of the measurements needed to achieve scientific goals of the study may preclude using inhalants and associated noise-generating equipment. Cats may be intubated and provided oxygen when pentobarbital is utilized. In consultation with RARC veterinarians, isoflurane (0.5-3.0%) anesthesia may be provided as an alternate to pentobarbital.

The acoustical noise created by some support and monitoring equipment may interfere with the acoustic measurements that are being studied, so a ventilator may be provided remotely if it can be ascertained that there is no noise interference from the equipment. Cats will be kept warm with appropriately-heated warming bags or other external heat sources positioned to safely provide supplemental heat, provided maintenance fluid support, monitored remotely via video, and with heart rate and O2 levels monitored continuously. Animals will be directly checked at least every 15 minutes.

A venous catheter is inserted for administration of fluids for all anesthetic events that are greater than 1 hour in duration, or as directed by an RARC veterinarian.

b. Are any paralytic agents being used? Underline one: **YES** **NO**
If YES, indicate agent, justification for use, and any special monitoring techniques used to assess animal condition while under paralysis.

28. Surgical Procedures

a. Describe the surgical procedure(s), including narrative description(s) for the following: reason for the surgery, incision site(s), tissue isolation methods, wound closure, and an estimate of time required to complete the surgery.
NOTE: *Aseptic procedures must be used for all survival surgery.*

See flow chart in 17a for surgical procedures timeline and information regarding assignment of animals to individual studies. Note that no individual animal undergoes all the potential procedures that are described in Q28a.
All implanted items are sterilized: cephalic implant post and screws by autoclaving, and more delicate items (eye and ear coils, cryoloops, and microelectrodes) by gas sterilization. All survival surgeries are done aseptically.

Surgery 1:
This procedure is performed under isoflorane/oxygen anesthesia to install an , and to place loops on the and in the external ears to monitor re placed by , placing the , securing with 7-0 Vicryl sutures or sterile surgical adhesive, and passing the end of the wire subcutaneously dorsally up to the for attachment to the This procedure has been previously used in this

laboratory (Yin and Greenwood, 1992a; 1992b; Populin and Yin, 1998a, 1998b, 1999). Likewise the [redacted] are placed [redacted] by an incision that is enlarged by blunt dissection so that a small coil of Teflon-coated stainless steel wire can be placed underneath the skin. The ends of the wire are also passed subcutaneously rostrally to the dorsal surface of the head where each wire is soldered to a connector embedded in the [redacted] Sutures are placed to close the [redacted] Each of the connectors is embedded in the [redacted] The use of a [redacted] is standard technique for neurophysiological studies of the [redacted]; this technique has significant advantages over the [redacted] in accuracy, stability and resistance to experimental artifact.

The [redacted] consisting of a stainless steel post, is [redacted] that in turn is attached to the underlying bone by 10-15 small titanium screws. In order to place the post at a precise angle on the head, the head is fixed in a stereotaxic instrument. An incision is made along the midline of the dorsal aspect of the head, and the overlying tissue and muscle attachments are carefully dissected free to expose an area of skull about 4 cm in diameter. To preserve the natural ear movements of the cat as much as possible, the muscle attachments will be disturbed as little as possible. The titanium screws are screwed or tapped into the bone, after which they are embedded in layers of [redacted]. Care is taken to ensure the screws do not pass through the full thickness of the bone and penetrate the cranial cavity. The skin caudal to the implant is closed with 4-0 monofilament sutures. The entire surgery for implanting the [redacted] takes approximately 6 hours.

If an animal has been previously trained or if training is not needed, then Surgery 1 may be combined with one of the other surgeries, such as the craniotomy and recording cylinder chamber implant of Surgery A.

[redacted]: Occasionally a loop of [redacted] may become displaced. Under light anesthesia a single suture or surgical anchor may be placed to re-secure the coil to prevent removal and additional replacement surgery. To replace a non-functioning [redacted] we will first remove [redacted] if it is visible and causing irritation to the cat, or from the subcutaneous space of the [redacted] is involved. Light anesthesia, e.g ketamine or ketamine/acepromazine as described in Q27a, may be used for [redacted] removal and/or removal of the associated connectors from the [redacted] The procedure for replacing the [redacted] is essentially the same as that for implanting the [redacted] except that the connectors for the previous coils may need to be removed from the [redacted] by removing the surrounding [redacted] replacements are done with the animal anesthetized as described in surgery1.

[redacted] repair/replacement: In the much more rare event of needing to repair or replace the [redacted] this procedure is also performed as described above. In the event that the [redacted] is gets loose and is displaced or comes off, the skull will be examined in consultation with RARC veterinarians to evaluate the health and integrity of the bone. Replacement of a [redacted] will only be performed after approval of an RARC veterinarian, and may involve closure of the implant site followed by an established minimum healing time prior to replacement.

Surgery A: [redacted]

This procedure comes several months later after the cat has been thoroughly trained, unless the cat has been previously trained. A [redacted] will be implanted on the skull under sterile conditions. In a non-invasive procedure, a discrete area of dental acrylic previously placed as part of the [redacted] will be removed either under previous sedation or on the day of surgery. With the animal under anesthesia, the head will be fixed in a stereotaxic apparatus. A midline incision will be made over the cranium and the skin and underlying tissue will be gently retracted. A craniotomy of 1.27 cm diameter will be trephined in the bone over the [redacted] (depending upon the experimental goals) [redacted] taking care not to touch the dura. A specially-made [redacted] used to hold the [redacted], is anchored over the craniotomy with [redacted] This surgical procedure takes about 3-4 hours.

Surgery B: [redacted]

To study the effect of [redacted] transected. Access to the nerves is accomplished by incisions along the caudal aspect of the pinnae that follow the course of the facial nerve. The relevant branches of the facial nerve that innervate the pinnae will be identified by electrical stimulation. The ear muscles are de-efferented by using a fine coagulator to transect the temporal and auriculoposterior branches of the facial nerve that innervate the pinna muscles. The incision is closed by monofilament sutures in the skin. In some cats pinnae will be denervated after behavioral training but before physiological recordings in order to study the behavioral effects of de-efferentation. Pinnae denervation will take place in other animals after recording from a sample of cells in order to study the physiological effects of de-efferentation. This procedure takes about 2-3 hours.

Surgery C: implantation of [redacted]

After animals are fully anesthetized, the dorsal surface of the head is shaved and stabilized in a stereotaxic frame using [redacted] The eyes are then covered with an opthalmic ointment to protect the corneas and keep them moist. A midline incision is made in the scalp, and a portion of the skull (approximately 8 to 10 mm diameter) is removed by a trephine in order to obtain access to the brain. Following incision of the dura mater [redacted] are permanently implanted in contact with the surface of the cerebral cortex. The [redacted] that are secured to the skull with surgical screws and dental acrylic. Subsequently, the dural margins are resutured with absorbable sutures and the skull piece replaced. Lidocaine (20 mg/mL) is injected subcutaneously at the edges of the incision (total dose not to exceed 500 mg), the incision closed with monofilament suture, and the cat is removed from the stereotaxic frame and allowed to recover under close supervision. The procedure to implant [redacted] takes approximately 6 hours.

Terminal perfusion

For animals in which electrophysiologic recordings have performed, location of electrode placement needs to be confirmed though histopathologic analysis. In these instances, a terminal perfusion of 10% formalin is performed at the time of humane euthanasia. The procedure for perfusion consists of initial induction with ketamine hydrochloride and acepromazine given intramuscularly. A lethal dose of sodium pentobarbital or other euthanizing solution is then administered IV (see Q21). Before the heart stops beating, but while the cat is under a deep plane of surgical anesthesia, the chest wall is opened and a cannula is inserted into the left ventricle of the heart to allow warmed saline (about 500 ml, or until the blood runs clear), followed by warmed 10% formalin (about 1000 ml), to perfuse the cardiovascular system.

b. Describe which of the following procedures will be used to maintain a sterile field during surgery (place an X between the brackets of all that apply):

[**X**] sterile instruments: specify method: [ ] bead sterilizer [**X**] autoclave [**X**] gas sterilizer (ethylene oxide):
[**X**] sterile gown/garb [**X**] sterile gloves [**X**] sterile drapes [**X**] face mask/eye protection
[**X**] surgeon scrub [**X**] other (please describe):(cetylcide II):

Sterile surgeries are performed in room 610 MSC of the LAR Animal Care facility. All instruments are sterilized in an autoclave, by gas sterilization using ethylene oxide gas, or by submersion in an antiseptic solution of Cetylcide II as per manufacturer's recommendation. Typically at least three people are involved in every surgery: two wear sterile gloves and gowns and the third maintains watch on the animal's condition and takes notes. All personnel wear a face mask and cap. The surgical site is aseptically prepped following removal of hair by 3 consecutive sets of betadine and alcohol scrubs.

29. Will the animals be allowed to recover from surgery? (Underline one) **YES NO**

If YES, describe the post-anesthetic and post-surgical monitoring and care procedures, including:

- all drugs and dosages
- how body temperature will be maintained during recovery
- the plan for suture or staple removal
- who will perform the monitoring, frequency/duration of monitoring
- the parameters that will be evaluated
- method of maintaining written records of these examinations
- measures designed to alleviate post-operative discomfort

NOTE: *Documentation of the post-operative monitoring of post-surgical animals is required!*

During the surgical procedure, heart rate, respiration rate, and body temperature are continuously monitored, and values recorded approximately every 15 minutes, from the time of induction until the animal has recovered from anesthesia and is returned to its cage.

Immediately after the surgery is completed, the anesthetic inhalant is turned off and medical-grade O_2 is provided through the endotracheal tube. The animal is kept on an external heat source and is monitored continuously by the anesthetist until extubated, then every 15 minutes until sternal and returned to its home cage. A cage-side heat lamp may be put in place during the overnight postsurgical period. Prior to surgery, cats are premedicated with buprenorphine (0.005-0.01 mg/kg) for sedation and analgesia. This will be repeated at 6 - 12 hours for any of the major procedures. Dosing at 6, 8, or 12 hours IM, SC or bucally will continue for 1-3 days or longer depending on procedure, animal response and direction from the RARC veterinary staff. To provide multi-modal pharmacologic analgesia, a nonsteroidal anti-inflammatory will be given, either ketoprofen (1-2 mg/kg SQ once daily for no more than 3 days) or, meloxicam (1st dose 0.1-0.2 mg/kg SC then oral 0.05 mg/kg PO q24h for 3-5 days) post-surgery or as recommended by an RARC veterinarian. Animals will be monitored at least once daily by the lab staff for a minimum of 3 days post-operatively.

Observations such as inappetence, hunched posture, failure to groom, or any other indications of pain or distress will be reported to the veterinary staff. The veterinary staff will be informed before surgeries are performed so that postoperative assessment of analgesia can be monitored and modified as recommended. All skin sutures or clips will be removed no later than 14 days post-operatively. Copies of the anesthesia and monitoring and a description of the surgical procedure will be placed in the animal's medical record.

Cefazolin (20 mg/kg, IM, IV or SQ), or other antibiotic as prescribed by an RARC veterinarian, is administered at the time of surgery and continued two or three times per day until the cat will take oral medications. At that point Orbax (5-7 mg/kg) or Cephalexin (20 mg/kg BID) will be dosed orally once daily for 1 week. If it appears that the cat will require more than one week of antibiotic, we will consult the veterinarians

30. Will any animal(s) be allowed to recover from more than one major operative procedure?
Underline one: **YES** **NO**

> **NOTE**: *A major operative procedure is defined as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.*

a. If **YES**, provide scientific justification for performing these procedures and list the species and number of animals:

Multiple survival surgeries are indicated for the protocol for the following reasons: First, the duration of the final electrophysiological recordings are limited by the hardening of the dura that occurs once the skull is exposed, which makes the dura too difficult to penetrate with a microelectrode. Since cats must be trained for many months before recordings can begin, it is necessary to complete the training before performing a craniotomy. In order to train the animals, we must first place the [redacted] so a surgical procedure is needed. If both procedures were done at once, the dura would be too hard before completion of the training. Thus, one surgery consists of implanting the [redacted] and a second surgery is needed for a craniotomy and to implant the [redacted] Second, [redacted] may break or become nonfunctional, which requires that they be removed and re-implanted. These cats will then undergo additional surgeries to replace the coils. Third, there may be some instances when a repeat craniotomy is needed due to regrowth of bone over the initial craniotomy. Fourth, in some of the [redacted] experiments the behavioral effects of [redacted] will be studied, which requires the initial surgery to implant the [redacted] so that the animal can be trained. Only after training is complete, which may require up to several months of time, can the [redacted] in a second surgical procedure. We endeavor to keep each of the surgical procedures as short as possible. Not all cats will be subjects in all experiments. As the flowchart in Q17a shows, typically all animals will undergo at least two major surgeries: **surgery 1** and either **surgery A, B,** or **C**. If an [redacted] repair is needed, additional procedures will be required. By using the cats for several experiments we are able to minimize the use of animals. We plan to use 3-5 cats/year.

As some of these animals may be on study for years, in order to promote the health and well-being of the animals, some animals assigned to this protocol may be spayed neutered by RARC veterinarians. All analgesia, anesthesia, and post-operative monitoring for this procedure will be done by the RARC veterinary staff. This surgical procedure will not be performed by the lab staff and is unrelated to the experimental use of these animals.

b. What is minimum length of time between the operative procedures?

Typically the minimum time between the initial **surgery 1** to implant the [redacted] and the next surgery (to implant the [redacted] etc.) is at least 3 months because the cat has to be trained during that period. The only reason for a shorter time between surgeries is if there is an equipment failure, such as the need to replace [redacted] In the case of any replacement or repair of the [redacted] RARC veterinarians will be consulted to ensure that animal's condition is suitable for repair or replacement of the implant.

Failure and/or breakage of [redacted] s the most common reason that multiple surgeries need to be performed. To estimate the failure rate o [redacted] the number of cats and time between surgeries, data from all of our past experiments was averaged. The failure rate of the [redacted] varied tremendously from 8 days to >2800 days so the mean number is not that meaningful. From 1998 to 2010 the median time between surgeries was 157 days. This does not accurately estimate the failure rate of [redacted] since it does not include cats that were euthanized with no [redacted] problem or those used in other components of the study. Nonetheless it does provide a rough estimate of the expected time between [redacted] replacement surgeries. If in any given cat this rate of [redacted] replacement becomes excessive, RARC veterinarians will be consulted to assess the situation and determine if further [redacted] replacement surgeries are possible. The minimum time between surgeries is 3 weeks.

Questions for Projects Using Wild-Caught Animals

(It is the responsibility of the PI to obtain all necessary state and federal permits for work with wild animals.)

31. Do you capture wild animals or do experimental manipulations (or procedures) on animals in the wild? Underline one: **YES** **NO**, Observation only

32. If you capture wild animals, describe how they will be trapped, what types of traps will be used, and how often traps will be checked.

33. **Quarantine and Release Information**

a. Describe quarantine procedures and precautions to prevent exposure of humans and other animals to zoonotic diseases.

NOTE: *If animals will not be housed, please state this.*

b. If animals will be released back to the wild, explain how the released animals will not present a disease exposure to wild populations and explain why this release will not expose the animal to greater risk of predation as a direct result of procedures performed or materials administered.

NOTE: *If animals will not be released back into the wild, please state this.*

34. If wild animals will be anesthetized and released to the wild, describe anesthetic doses, method of administering and procedures for assuring that animals are sufficiently recovered from anesthetic to be released. Consider that prey species may have to be monitored until fully recovered to avoid predation.

NOTE: If animals will not be anesthetized, please state this.

Questions for Projects Using Nonhuman Primates

35. **Nonhuman Primate Enrichment**

a. If nonhuman primates used in your study must be housed individually due to scientific consideration, provide that scientific rational.

b. Provide scientific rationale for any restrictions to environmental enrichment. Include the specific restriction(s) such as: puzzle feeders, cage perch, wooden chew sticks, food treats (bananas, carrots, oranges, other fruit or vegetables), etc.