# The University of Mississippi Medical Center

## Animal Activity Protocol

IACUC - Institutional Animal Care and Use Committee
Telephone [redacted] / Facsimile [redacted]
iacuc@umc.edu

## Contents

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## To be completed by IACUC

| Protocol Number: **0936F** | Date: **04/09/2020** | Classification: **D** |
|---|---|---|

## 1. Principal Investigator

| | | | |
|---|---|---|---|
| **Name** | [redacted] | | |
| | **☒PhD ☐ MD ☐ Other:** | | |
| **Title** | **Professor** | | |
| **Dept.** | **Otolaryngology and Communicative Sciences** | | |
| **Phone #** | [redacted] | **Office Location** | [redacted] |
| **email** | [redacted] | **Emergency #** | [redacted] |

**Note: The emergency number should be a number at which the PI can be contacted on nights and weekends.**

## 2. Other Personnel

**All listed personnel must complete IACUC required training, including completion of Occupational Health forms and submit a Training Requirements Registration form prior to working with animals and receiving access into the Center for Comparative Research (CCR).**

**You may authorize personnel to submit modifications to this protocol by checking the box for signing privileges.**

| Name | Title | Ext/Cell | Email | Signing Privileges |
|---|---|---|---|---|
| [redacted] | Researcher IV | [redacted] | [redacted] | ☐ |
| [redacted] | Researcher III | [redacted] | [redacted] | |
| [redacted] | Professor | [redacted] | [redacted] | |
| [redacted] | Scientist I | [redacted] | [redacted] | |
| [redacted] | Professor | [redacted] | [redacted] | |
| [redacted] | Professor | [redacted] | [redacted] | |
| | | | | |

*(Insert additional lines as needed)*

## 3. Project Title:

Neurophysiology of vestibular and oculomotor functions

## 4. Proposal is **3 year Full Submission Renewal (must attach Appendix K)**

## 5a. Outside Contracts

Will any components of this study involve live animals maintained at another institution?

☒ No

☐ Yes (if yes, provide information on the level of involvement)

## 5b. Animal Behavior Core

Will this study use the Animal Behavior Core (ABC)?

☒ No

☐ Yes – Requires review and approval by ABC Director. See Appendix L.

## 6. Funding Source

☒ Extramural/Intramural Funding

| | | | |
|---|---|---|---|
| Title | Neurophysiology of vestibular and oculomotor functions | | |
| PI | [redacted] | | |
| Funding Agency | NIH/NIDCD | | |
| Status | ☐ Submitted ☒Funded | Grant Number | R01DC014930 |
| Covered Dates | 09/01/2019-08/31/2022 | | |

*(Copy and paste table if project is funded by multiple grants.)*

☒ Department – List Department: Otolaryngology and Communicative Sciences

☒ Other (*Example: Divisional funds which you have control over, start-up funds*)

Explain:

Startup/Incentive funds

## 7. Dates of Study

Anticipated start date of study: 4/17/2020

**All investigators must adhere to a federally mandated three-year cycle of full protocol review, even if a funding period exceeds three years in duration.**

## 8. Source of Animals

Will any animals be obtained from non-commercial sources? ☒No ☐Yes

If Yes, list:

**Note: Animals from non-commercial sources must have their health status evaluated by a CCR veterinarian prior to their arrival at UMMC. This question does not relate to the acquisition of animals from other UMMC investigators. If animals are transferred from a UMMC source, an Animal Transfer Form must be completed and approved for each transfer.**

## 9. Animal Requirements

For **New** submissions complete **Table A.**
For **3 Year FSR** submissions complete **Table B**.

Animal numbers MUST be calculated for a period not to exceed three (3) years from the start of the study.

### A. New:

| Species | Strain/stock | Sex | Source | Total for 3 years | Average daily census |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

*(Insert additional lines as needed)*

Note: If using nonhuman primates, complete Appendix A.

### B. 3 Year FSR: For a 3-year renewal, number of animals needed to complete the studies in this protocol. This must include the number of animals to be received plus the number of animals currently on campus to be carried over from the previous version of this protocol.

***Example:*** You need 100 animals to complete your study and you have 20 animals currently in house to carry over to this is protocol.

| *Total Needed for 3 years* | | *Total Carried Over* | | *Total Requested* |
|---|---|---|---|---|
| 100 | - | 20 | = | 80 |

You will be approved for 100 animals to complete the study (number to be justified in question #17) of which you already have 20, so you will have 80 animals available to order.

| Species | Strain/stock | Sex | Source | Total Needed For 3 years | Total Carried Over | Total Requested (Needed – Carried Over) | Average daily census |
|---|---|---|---|---|---|---|---|
| Macaca Mulatta | | M/F | Varied | 7 | 4 | 3 | 4 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

*(Insert additional lines as needed)*
***Originally approved for 7; 4/9/20***

**Note: The number of animals available for ordering will be the difference between total animals needed minus carryover animals.**

**C.** List any unusual phenotypes or abnormalities associated with the animals (including sublines) listed above (i.e., prone to diarrhea, decreased appetite, patchy hair loss, increased sensitivity to pain, slow wound healing, etc.).

## 10. Breeding program

Will animals be involved in a breeding program at UMMC or will time-pregnant animals be used?

☒ No

☐ Yes (if yes, provide information in Appendix B)

## 11. Potential Hazards

| | | Yes | No | Pending |
|---|---|---|---|---|
| **A** | **Chemical toxins used in animals?** | ☐ | ☒ | |
| | **Reviewed by Environmental Health & Safety?** | ☐ | ☒ | ☐ |
| **B** | **Radioisotopes used in animals?** | ☐ | ☒ | |
| | **Reviewed by Radiation Safety?** | ☐ | ☒ | ☐ |
| **C** | **Use of laser, CT, x-ray, or fluoroscopy?** | ☐ | ☒ | |
| | **Reviewed by Radiation Safety?** | ☐ | ☐ | ☐ |
| **D** | **Biohazards used in animals?** | ☒ | ☐ | |
| | **Reviewed by Institutional Biohazard Committee?** | ☒ | ☐ | ☐ |
| **E** | **Human cells used in animals?** | ☐ | ☒ | |
| | **Reviewed by Institutional Biohazard Committee?** | ☐ | ☒ | ☐ |

If YES, provide specific details of specialized animal husbandry, care, cleaning, or decontamination procedures**, especially identifying responsible parties**.

The biohazard is herps B virus. Lab staff ([redacted]) have been trained to be in compliance with protocols of handing the NHP with a BSL-2 status. BSL-2 warning signs are posted in the lab. First-aid kits and eye-washers are available in the lab. Protective clothes, gloves, masks and eye glasses are required for handling the NHP.

## 12a. Animal Husbandry

| | Standard | Nonstandard |
|---|---|---|
| **Feeding** | ☒ | ☐ |
| **Watering** | ☒ | ☒ |
| **Caging** | ☒ | ☐ |
| **Room/Environment** | ☒ | ☐ |
| **Altered light cycle** | ☒ | ☐ |

Note: Provide complete explanation and justification for any **nonstandard animal husbandry** (e.g. metabolic caging, restraint chairs, transport devices, singly housed animals, altered light cycle). Protocols listing non-standard husbandry must provide complete details of

the cleaning and sanitation, **especially identifying responsible parties**:

**In the period when animals are not for testing**, they will have automatic water in the home cage on weekdays and weekends.

**In the period when animals are for testing**, they will not have automatic water in the home cage. Instead, they will obtain most of their fluids in the lab. Animals are trained to fixate visual targets for juice rewards. In a typical trial, a monkey is required to maintain fixation on a visual target for about 0.5-1 s to obtain 2 drops of apple juice (~0.2ml). Monkeys can have as much liquid as they can during an experiment session as long as they maintain fixation on the target presented to them. In a typical day, monkeys usually finish about 2 to 3 thousand trials within 2~4 hours to get about 300ml fluids, which is adequate for the monkey to stay hydrated (about 30ml/kg/day, or 2400ml/day for a 80kg human). While we do not have an upper limit of fluid intake, we do make sure the monkey have at least 30ml/kg. In days when fluid intake is less than 30 ml/kg in the lab, the monkeys will be given the balance amount of fluid in the laboratory or in the home cage. We also make sure additional 50ml liquid will be provided in the home page to help monkeys eating food. On Fridays, lab staff will provide a full bottle of 1000ml water in the home cage. On Saturdays, Sundays and holidays, the CCR staff will provides a full bottle of 1000ml water or re-attach water line. To ensure individual access to water, monkeys may need to be separated upon consultation with the CCR.

In weekdays (Monday-Friday), records of fluid, food consumption and body weights are maintained in the lab by the lab staff ([redacted]) during studies. If a monkey is found to have a loss of body weight over 10%, we will consult CCR immediately to assess the status of the monkey to determine whether it needs to have access to automatic water. The water scheduling/food intake are consistent with the APV Guidelines, and water records/documentation are kept with each animal.

## 12b. Singly Housed Animals

Will animals be singly housed?

☐ No

☒ Yes – Please provide justification for single housing. **NOTE: If using non-human primates you must complete Appendix A.**

Our studies do not request animals to be singly housed at any time during the studies. The CCR has total discretion to arrange the housing at all times. Although the protocol does not request monkeys to be singly housed, it is possible that the CCR sometimes may not find a compatible mate for a monkey. Thus, the monkey may be singly housed due to lack of a compatible mate.

## 13. Housing

Will animals be housed outside of the CCR for greater than 12 hours?

☒ No

☐ Yes Where?

Note: If yes, provide complete explanation and justification for any **decentralized animal housing.**

## 14. Objectives in lay terminology

In **non-technical**/lay terminology, what is the **objective of the experiments** proposed in this Animal Activity Protocol? (i.e. ***Response should be written in non-scientific language, as though explaining the study to a high school student***.)

- In non-technical**/lay terminology**, what is the objective of the experiments proposed in this Animal Activity Protocol?
- Why are the experiments proposed?
- What knowledge do you hope to achieve?
- What is the potential relevance (e.g. benefits) of experimental findings to human or animal health, advancement of knowledge, and/or the good of society?

Generally, **single sentence explanations** for these types of questions will suffice.

This project studies how the brain processes sensory information regarding object motion and self-motion. Deficits in this function resulting from diseases severely impair our ability to see and maintain balance during motion. Our goal is to search for adaptation mechanisms that overcome these deficits. In this project, we are going to study eye movement behaviors and neuron activities of monkeys during visual motion, whole-body motion and voluntary movements. This study will provide important knowledge for understanding fundamental vision and balance mechanisms and improving diagnosis and treatment of balance disorders in humans.

## 15. Rationale

**A.** What is the rationale for using animals rather than using non-animal models?

Our goal is to understand how an intact neural system responds to natural environmental stimuli with appropriate motor responses; thus, anesthetized or in vitro models cannot be used. Because the research is invasive, involving recording of neuronal activity and electrical stimulation within the central nervous system, human subjects cannot be used.

**B.** What is the rationale for using the particular animal species and/or strain noted in Item 9?

Previous research has shown that the macaque monkey's visual, vestibular and oculomotor systems are essentially identical to those of humans. The neuroanatomy and neurophysiology relevant to this research has been extensively worked out in these species. For these reasons, the monkey is an excellent animal model for investigating eye movements and vestibulo-ocular reflexes. The experiments also depend on animals reliably performing visual tracking tasks. Monkeys excel in this behavior. In addition, over the past 25 years, I have recorded and published behavioral and neural data from macaque monkeys that provide essential control data for these experiments.

## 16. Brief Outline

**Provide a general description of the animal procedures included in the experimental design. This description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study.**

- **Briefly outline** the proposed animal manipulations and provide a time-line of events.
- **Note that specific details about methods and procedures will be required in the appropriate appendix (see list below)**

- **Complete** only those **appendices that apply** to the animal manipulations in your experimental design.
- If possible, flow charts and/or time lines should be included to clarify the timing of procedures which are to be performed.

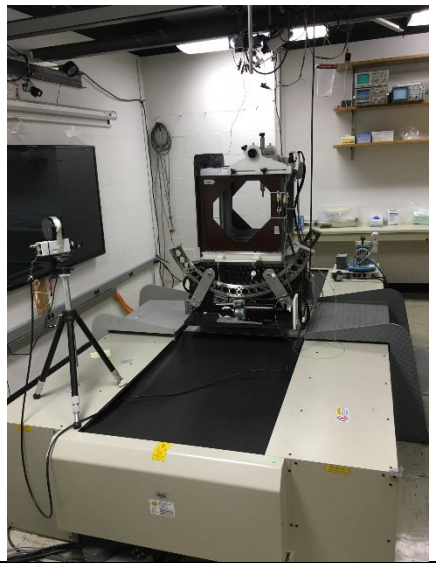
Verbatim descriptions from a grant submission are not acceptable and will not be reviewed.

The animal research protocol involves (a) animal handling and food/water intake assessment procedures, (b) surgical procedures for recording eye movement and single unit activity, and (c) experimental procedures for data acquisition.

**(a) Animal handing procedures**

*Chair Training*

Each monkey is fitted with a collar that can be hooked to a pole with a clamping hooked end. Using fruits as rewards, monkeys are trained first to come to the front of the cage, then out of the cage on the floor, then to the monkey chair that fits its size. The monkey is returned to the home cage in a similar fashion. Monkeys need to complete this training before any surgical procedures.



The primate chair is designed to provide adequate space for monkeys with various sizes to sit comfortably. We will consult the CCR staff to evaluate whether a monkey is too big for the chair. Chairs will be modified or new chairs will be made to accommodate oversized monkeys.

*Animal head support*

For recording eye movement and single unit activity during eye tracking tasks, the monkey will be seated in the primate chair (shown in the above photo), which will be secured to the platform of a vestibular stimulator within the frame shown in the right photo. The monkey's head is held in a natural upright position supported by a face mask (shown in the right photo) made from Plexiglas and padded with moleskin that fits snugly across the snout and a u-shaped neck/head collar piece positioned to the back of the head to prevent withdrawal from the face mask. Each monkey will have its own mask pads.

*Behavioral training and water/food intake assessment*

After a monkey completes the chair training, it will be ready for behavioral training in the lab. The monkey will be trained to visually track a stationary or moving target for juice rewards while it may be rotated/translated or making voluntary head movements. Their eye positions will be measured by the search coil technique or a video-based eye tracker. Monkeys can have as much liquids as they want as long as they maintain fixation on the visual targets presented to them. Records of water consumed in the laboratory, food intake in the home cage and body weights will be maintained during studies. Monkeys' behavioral performance in the lab, general behavior in the cage and status of head implants will be assessed with the CCR.

*Neurophysiological recording*

We will use two approaches to record electrical signals from animals. One is to use surface or intramuscular electrodes to record myogenic potentials from eye muscles and neck muscles. The other one is to record extracellular action potentials from neurons located in the monkey's peripheral and central nervous system. This procedure is standardized and employed in laboratories worldwide. Neurons are recorded using microelectrodes (tip sizes of the order of 1~10 microns) introduced into the brain through a recording well and a guide tube and advanced using a hydraulic microdrive. Except for the initial puncture of the dura by a cannula, the procedure does not cause animal discomfort as brain tissue is insensate. This is also evidenced by their continuing to work on the task while the electrode is advanced in the brain. To minimize the discomfort of dura puncture, which is likely to be similar to a quick needle stick, the site is anesthetized with a drop of lidocaine. A separate cannula is used for each monkey. The cannula is cleaned on the weekdays, and soaked in nolvasan (diluted 12:500) in a jar for at least 30 minutes and then rinsed with sterile saline before being introduced into the brain. The nolvasan in the jar is changed on the weekdays. The nolvasan in the bottle is replaced within a couple of months. The electrodes are rinsed by sterile saline before and after each use. They are too delicate for sterilization treatment. However, our experience (25 years), and that of our colleagues in other institutions, are that electrode introduced infection is extremely rare. We will consult the CCR staff to implement the sanitization process.

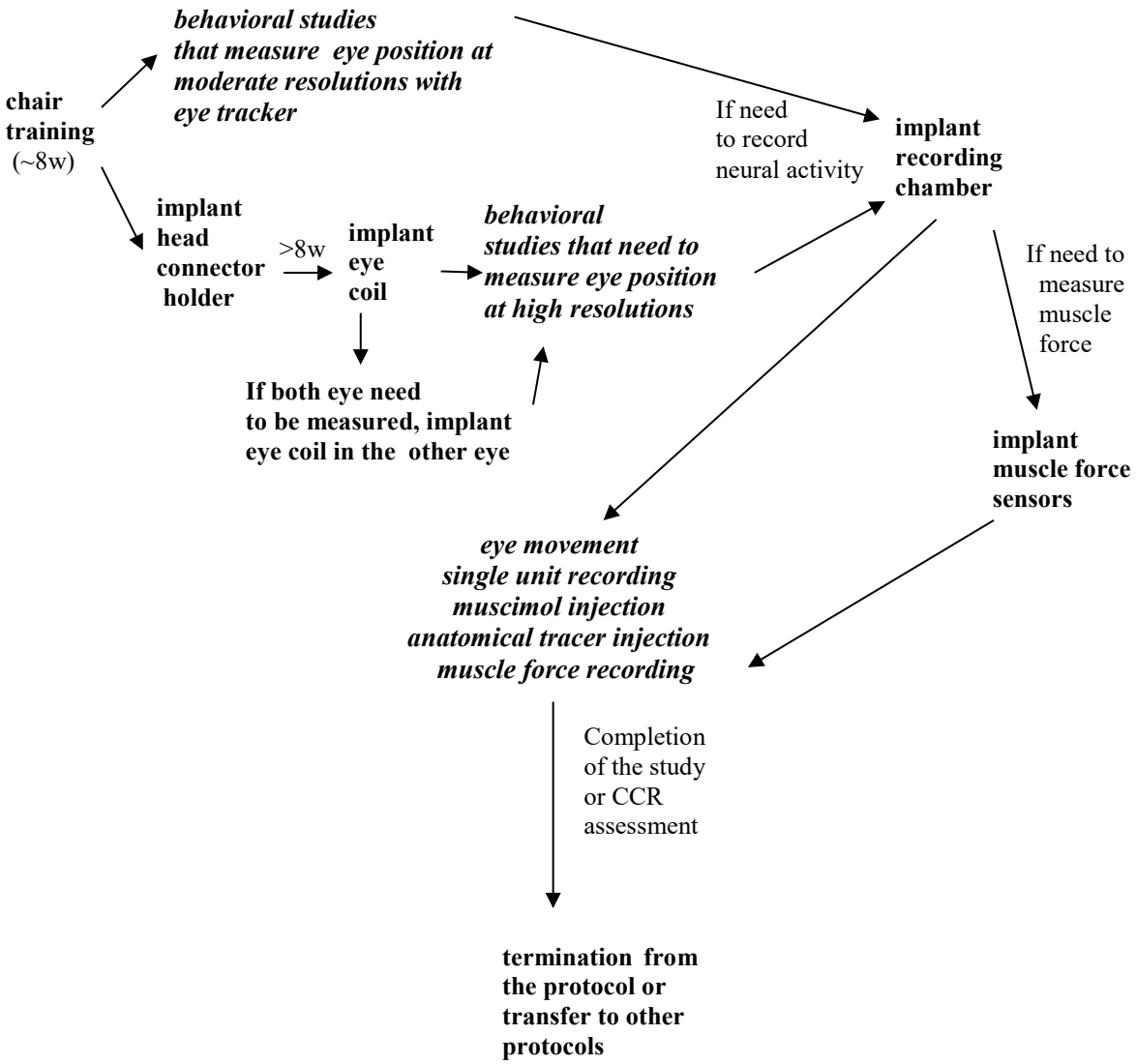
**(b) Surgical procedures.** For behavioral experiments that only need to measure eye position at moderate temporal and spatial resolutions, a video-based eye tracker is adequate and monkeys will not need any surgical procedures.

For behavioral experiments that require measuring eye position at high temporal and spatial resolutions, monkeys will need surgical procedures for implanting a head connector holder and an eye coil in each eye (Appendix C).

For experiments that need measuring singly unit activity, monkeys will need the surgical procedure to implant one or two recording chamber.

For experiments that need measuring muscle force, monkeys will need the surgical procedure to implant two muscle force sensors in the medial and lateral rectus muscles.

In addition to single unit recording, we may also apply muscimol to inactivate the identified target areas to examine their functions. Upon completion of the study, anatomical tracers will be injected into the target areas for histology and neuroanatomical studies. Monkeys will be then euthanized and tissues will be harvested.




If monkeys cannot be tested due to untreatable healthy conditions or inability to perform any behavioral tasks, monkeys will be euthanized upon consultation with the CCR or transferred to other protocols.

Note that the above flow chart is not describing special scientific experiments. They are the surgical procedures that are needed to conduct behavioral and neurophysiological studies. The time intervals related to each procedure are estimated based on our experience and may change upon consultation with the CCR staff.

**(c) Experimental procedures**

All monkeys will experience a similar regimen. Monkeys are trained to enter the chair with minimal efforts. Animals are transported from the housing area to the laboratory in a closed transport container. In the laboratory the monkey chair is placed into the experimental apparatus and the monkey's head is supported by the face mask and head implants are examined and cleaned prior to the beginning of a session to prevent/reduce contamination.

Each experiment may have two components. One is to study eye movement responses to various sensory stimulation, including visual images, acoustic waves (clicks, tones, noise, up to 100 dB NHL), passive head rotation (peak speed 150deg/s), passive head translation (peak acceleration 0.4G), or active head movement. The other one is to correlate the neuronal responses to the eye movement responses in these conditions. Visual stimulation is presented on a tangent screen or a TV monitor in front of the monkey. Acoustic waves will be generated by an auditory amplifier (Tuck-Davis Technologies) driven by voltage signals generated by a CED device (Cambridge Electronic Design). The sound stimulation (up to 100 dB NHL) are safe to be administrated to monkeys via an ear-insert phone.

In an experiment session, the computer initiates the behavioral task and rewards the monkey for tracking a visual target with or without head movement. The sensory stimulation is controlled by the computers. Single unit activity may be recorded from neurons in the brainstem, cerebellum, cortex or peripheral nerves (e.g., vestibular/auditory afferents). During some instances, weak current pulses (10~50 uA, 0.1ms duration) or small amount of chemicals (muscimol and anatomical tracer) may be injected through the microelectrode or the cannula to the recording sites. The monkey is generally unaware of the microstimulation and injections. Electrical stimulation will activate the neurons near the tip of the electrode so their roles in motor control can be investigated. Injection of muscimol will temporarily inactivates a small region of the nervous system and allow us to examine its role in gaze control. Neuroanatomical tracers (e.g., BDA and WGA-HRP) injected into the recorded region in the last recording session will help identify the inputs and outputs of the region. At the end of the experiment, the animal is returned to its home cage.

## 17. Justification of animal number

Explain and **justify** how the number of animals requested was determined. (Flow diagrams/tables to define animal use are encouraged. **Statistical support should be included.** This number should support the request made in the *Total for 3 years* column in #9 and be consistent with the outline in #16).

Our projects require large numbers of single unit recordings be made from several sites, including brainstem, cerebellum, cortex and peripheral nerves. We will need about 100 complete sets of recordings to adequately describe and characterize each population of cells. At least 700 cells are needed to achieve our goals. Two monkeys may be run each day and good recordings will be obtained in 20~30% of the time. We normally keep 3 monkeys active for the project. Previous experience suggests that about 100 well characterized cells could be

obtained from one animal in about 3 years. Thus, a minimum of 7 monkeys will be needed for the project. A total of 7 monkeys are requested for the three years. Whenever possible, animals will be used initially in behavioral studies, and for recording from as many sites as is reasonable with respect to the project goals and the animals' well-being.

## 18. Location & transportation

**A.** Indicate room(s) where animal procedures will be conducted.

| Room Number | Procedures performed |
|---|---|
| [redacted] | Behavioral and physiological recordings |
| [redacted] | Behavioral and physiological recordings |
| [redacted] | Surgeries |

*(Insert additional lines as needed)*

**B.** Studies involving animal transportation to locations other than the housing area must identify the animal transport device, the nature of the shrouds used to cover the transport device, and describe the route of transport. **Include transport within the CCR (e.g. IVIS, surgery room).**

Monkeys are housed in the [redacted]). For testing, they will be transported through corridors and elevators (25 or 26) to [redacted]). Monkeys will be seated in primate chairs and transported by a metal cart that is fully covered. For surgeries, they will be draped with a sheet and transported from the home room ([redacted]) to [redacted] within the CCR on a cart.

## 19. Euthanasia

**A.** At what point in the proposed experiments will animals normally be euthanized, (experimental end-points)? Or at what point will any individual animal be euthanized?

The CCR veterinary staff works closely with NHP populations, and that CCR vet staff does daily checks on each NHP M-F. Behavioral and neuronal studies may continue as long as a monkey is in general good health and suitable for the experiments. Euthanasia is performed when the histology needs to be performed on brain tissues.

**B.** What humane endpoints or criteria will be used to determine if an animal is to be euthanized prior to, rather than at, the anticipated end-point of an experiment? Note: Contact CCR, ext. [redacted], for recommendations on the assessment criteria.

Monkeys' health status will be regularly evaluated. If their health is compromised as indicated by loss of weight about 10%, appetite and interests in behavioral tasks, the CCR will be contacted immediately and monkeys will be treated by the CCR and returned to the study after a full recovery. If a monkey has untreatable healthy conditions and become unsuitable for the experiments, we will consult the CCR to either euthanize the monkey or transfer the monkey to other protocols.

**C.** Will natural death (or death due to manipulations) be used as an endpoint?

☒No ☐Yes – if "Yes", explain and justify.

## 20. Euthanasia Procedures

What procedures will be used to euthanize the animals? Note: Secondary methods are required to ensure death. (Consult the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition for appropriate methods of euthanasia or contact the CCR.)

A deep surgical plane of anesthesia will be achieved by ketamine induction (10 mg/kg, IM) followed by sodium pentobarbital, 50mmg/kg, IV until loss of corneal and withdrawal reflexes. After the surgical plane of anesthesia is attained, the animal is euthanized by transcardiac infusion of fixative (formalin). Brain tissue will be collected to perform histology for locating recording sites. This also ensures the death of the terminated animals. The CCR will be consulted for the euthanasia procedures. PPE will be used when handling formalin.

## Assurances

1. Have all personnel received a medical evaluation from UMMC Student/Employee Health and updated Occupational Health Information annually?

   ☐No ☒Yes

2. Have all personnel listed on this protocol been informed and understand their role in the experiments?

   ☐No ☒Yes

3. Review of the available resources and previous experiments have determined that the proposed activity is not unnecessarily duplicative of previously reported activities.

   ☐No ☒Yes

**USDA Policy #12, "Consideration of Alternative to Painful/Distressful Procedures": states the following:** ***The Animal Welfare Act (AWA) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including refinements, reductions, and replacements.***

**List each potentially painful or distressing procedure included in these protocol:**

| | |
|---|---|
| Head implants | Eye coil implantation |
| Fluid restriction | |

To comply with Policy #12, investigators are required to conduct literature searches using **two different search engines (see below)** addressing each of the procedures listed above. Specific procedures listed may be utilized as key terms.

Additional assistance may be obtained by contacting the Rowland Medical Library reference desk at ext. [redacted]. See IACUC Guidance on Minimizing Pain and Distress in Animals and Searching for Alternatives.

## Helpful Databases

(Please note: PubMed and Medline are the same and cannot both be used.)

☒Medline/PubMed (http://www.ncbi.nlm.nih.gov/pubmed)
☐Toxnet (http://toxnet.nlm.nih.gov)
☐AWIC (http://awic.nal.usda.gov)
☐Agricola (http://agricola.nal.usda.gov)
☒Scopus (http://www.scopus.com/home.url)
☐Other (Click here to enter text.)

| Name of the database | Date of search | Period of years covered by the search | Potentially painful or distressing procedures addressed | Key words and/or search strategy used | Indicate which mandate each search addressed | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Replacement of animals | Reduction in numbers of animals used | Refinement to minimize pain or distress | Lack of unnecessary duplication |
| Medline | 01/30/2020 | all | Eye coil implantation | Eye tracker, non-invasive eye tracking | ☐ | ☒ | ☒ | ☐ |
| Medline | 01/30/2020 | all | Head holder implant | Non-invasive head holding, restraint, | ☒ | ☒ | ☒ | ☐ |
| Medline | 01/30/2020 | all | fluid restriction | fluid restriction, monkey, eye movement | ☐ | ☐ | ☒ | ☐ |
| Medline | 01/30/2020 | all | | Alternative animal model, vestibular, oculomotor | ☒ | ☐ | ☐ | ☒ |
| Scopus | 01/30/2020 | all | Eye coil implantation | Eye tracker, non-invasive eye tracking | ☐ | ☒ | ☒ | ☐ |
| Scopus | 01/30/2020 | all | Head holder implant | Non-invasive head holding | ☐ | ☒ | ☒ | ☐ |
| Scopus | 01/30/2020 | all | fluid restriction | fluid restriction, monkey, eye movement | ☐ | ☐ | ☒ | ☐ |
| Scopus | 01/30/2020 | all | | Alternative animal model, vestibular, oculomotor | ☒ | ☐ | ☐ | ☒ |

**Narrative**

*Below, provide a brief summary of any articles that were identified in the search and how these studies relate to the current animal protocol. The narrative must discuss what efforts were made to REDUCE animal number and REFINE experimental procedures to reduce or eliminate pain and distress to the experimental animals, as well as whether there are alternatives that could REPLACE the use of animals. Interaction with peers and educational*

*materials may be used to supplement discussion of literature searches.*

Summary of articles:

The literature revealed important publications on using video-based eye trackers to monitor eye movement and non-invasive methods to support monkeys' head during behavioral and neurophysiological recordings. However, the search did not reveal publications to replace the monkey model. Instead, the research has shown that the monkey is an excellent animal model to study gaze control in various conditions for three reasons. First, the macaque monkey's visual, vestibular and oculomotor systems are essentially identical to those of humans. Second, the neuroanatomy and neurophysiology relevant to this research has been extensively worked out in these species. Third, the experiments also depend on the animals reliably performing difficult visual tracking tasks. Monkeys excel in this behavior. In addition, over the past 25 years, I have recorded and published behavioral and neural data from macaque monkeys that provide essential control data for these experiments.

**Reductions** in animal number:

Presently, each experiment will be conducted in at least two monkeys. This is to meet the minimal requirements for repeatability by most peer-reviewed journals. Additional monkeys are requested to be prepared to replace the monkeys that will be terminated upon completion of their studies or unexpectedly due to illness.

**Refinements** to methods to reduce distress:

We did not find publications on alternatives to fluid restriction for eye movement studies in monkeys. However, we identified three areas of refinements to reduce distress. First, we will try to limit uses of squeezing the back of the cage to get a monkey to the front door to connect its collar to a pole. We will introduce treats to encourage the monkey to come to the front door voluntarily. It will take about two months for the monkey to learn this protocol. Second, while the eye coil technique is still essential for experiments that need measuring eye position at high resolutions, for experiments that do not need measuring eye position at high resolutions, we will setup a video-based eye tracking system. This will eliminate the need for two head implant procedures (i.e., implanting a head connector holder and an eye coil). Over the years, we have identified the experiments that only need sampling eye position at moderate resolutions and also identified an eye tracker for this purpose. We will try it in the coming years. Third, we will employ the face mask approach to support the head during experiments. This non-invasive approach will replace the previous approach using a head holder implant. While we will still need to implant a head connector holder to anchor the eye coil connector, the connector holder is much smaller and will likely stay longer as it will not be used to restraint the monkey head and gradually get loose due to the mechanical stress.

Animal **Replacement**:

The searches found no articles that provided animal replacement.

## Training and Qualifications

- PI

Name► [redacted]
Animal research experience ► 25 years

Qualifications to perform specific procedures

| Specific procedure(s) that the PI will perform personally | Experience with each procedure in the species described in this Protocol |
|---|---|
| Eye coil/head post/recording chamber | 25 year experience of the procedures |
| Behavioral testing and recording | 25 year experience of the procedures |

- Other research personnel (copy the lines below for each individual listed as personnel on protocol)

Name► [redacted]
Animal research experience ►15 years

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this Protocol |
|---|---|
| Behavioral testing and recording | 15 years |
| Animal care | 15 years |

Name► [redacted]
Animal research experience ► 16 years

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this Protocol |
|---|---|
| Behavioral testing and recording | 16 years |
| Animal care | 16 years |

Name► [redacted]
Animal research experience ► 8 years

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this Protocol |
|---|---|
| Behavioral testing and recording | 6 years |
| Animal care | 6 years |

Name► [redacted]
Animal research experience ► 23 years

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this Protocol |
|---|---|
| Head connector holder | 3 year experience of assisting and conducting the procedure |
| | |

Name► [redacted]
Animal research experience ► 15 years

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this Protocol |
|---|---|
| Eye coil | Certified eye surgeon in UMMC and 2year experience of the procedure. |
| | |

Name► [redacted]
Animal research experience ► 30 years

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this Protocol |
|---|---|
| Muscimol and tracer injection | 15 year experience of the procedure |

- **Training to be provided.** List here each procedure for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone, enter "N/A"

| N/A |
|---|

## Certification of the Principal Investigator:

Signature certifies that the Principal Investigator will conduct the project in full accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, USDA regulations, and UMC policies governing the use of live vertebrate animals for research and teaching purposes. The procedures involving animals will be conducted by trained or experienced personnel or under the direct supervision of trained or experienced persons. It is understood that IACUC approval is valid for a period of 12 months following the date of original approval and must be renewed annually for continued approval. I understand there is a 3-year requirement for full protocol rewrite. It is further understood that should this project be submitted for external funding, the information presented on the UMMC Animal Activity Protocol form accurately reflects the animal use in the full grant application.

[redacted]

X_____

Signature of Principal Investigator (Paste digital copy of signature)

### Approval by the Attending Veterinarian:

### Approval by the Institutional Animal Care and Use Committee:

Routed through SharePoint:

| Assigned To | Title | Due Date | Status | Related Content | Outcome |
|---|---|---|---|---|---|
| ☑ | [redacted] | Please approve FSR_0936 | Completed | FSR_0936 | Approved |
| ☑ | [redacted] | Please approve FSR_0936 | Completed | FSR_0936 | Approved |

| **Appendix A** | **Non-Human Primate Environmental Enhancement/Enrichment** |
|---|---|

This appendix must be appended to each protocol involving the use of nonhuman primates.

Nonhuman primates must have their physical environments enhanced/enriched by providing means of expressing non-injurious, species-typical activities. The *Animal Welfare Act* (9 CFR 3.81) states that research facilities "must develop, document, and follow an appropriate plan for environment enhancement adequate to promote the psychological well-being of nonhuman primates".

The default position of USDA and OLAW is that non-human primates must be socially housed. The Guide (2011) states, "*… nonhuman primates should normally have social housing (i.e., in compatible pairs or in larger groups of compatible animals)".* Exemptions to the social housing requirement must be based on **strong scientific justification** approved by the IACUC or for a specific veterinary or behavioral reason.

***The Center for Comparative Research provides an active plan of environmental enrichment that includes cage complexities (tunnels, barrels), social interaction, fruit/vegetable supplements, foraging, and manipulative devices/toys. Unless otherwise, specified, the CCR will provide all available forms of enrichment.***

## 1. Enrichment Techniques

Are there any enrichment forms/techniques that are included in this protocol?

☐No ☒Yes

## 2. Description

Describe the above techniques.

In the lab, juice rewards are provided for performing behavioral tasks (i.e., fixating or tracking a visual target). During the initial training, fruit rewards will be provided for accepting the hook and sitting in the chair. The monkeys also receive the forms of enrichment provided by the CCR. We will work with the CCR to develop additional forms of enrichment and to address social needs of individual monkeys.

## 3. Exemption from Enrichment

Are there any forms of enrichment/enhancement that should not be used in this study?

☒No ☐Yes

## 4. Justification for Exemption

If Yes, provide complete justification for this exemption.

**Exemption from social/pair housing:**

**Exemption from other forms of enrichment:**

## Appendix B Time-Pregnant/Breeding Programs

Complete Appendix B for all proposals planning on establishing a breeding colony or for those studies utilizing time-pregnant animals. Studies incorporating breeding programs or offspring from time-pregnant animals will be required to report annual production (number of offspring used) at the time of IACUC protocol annual renewal.

### 1. Description

a. Provide a specific description of the type of breeding program to be utilized (monogamous pair, "trio" breeding: 2 females and 1 male, "harem" breeding: up to 4 females and 1 male, etc.).

b. The *Guide for the Care and Use of Laboratory Animals* sets minimum space requirements for breeding animals. *See chart below.

If you wish to request a deviation from the minimum requirement provide justification based on performance standards (e.g., health, reproduction, growth, behavior, activity, and use of space) and special needs determined by the characteristics of the animal strain or species (e.g., obese, hyperactive) and experimental use (e.g., animals in long-term studies may require greater and more complex space).

c. For all mating schemes other than pair breeding, pregnant females must be separated prior to birth of the litter unless an exemption is justified. If using trio or harem breeding, please describe how/when dams will be separated to ensure that overcrowding does not occur.

d. All litters must be separated at 21 days of age unless an exemption is justified. Please describe specific plans for weaning.

### 2. Personnel Responsible

Identify personnel responsible for the breeding program, including weaning and documentation of program.

## 3. Records

Please describe the record-keeping system that will be used and how breeding, health and maintenance of the colony is recorded.

## 4. Adults

a. How many adults will be utilized in this breeding program over the 3 year period?

b. How many breeding pairs/groups will be utilized at one time (may be explained with a range)?

c. How many breeding cycles will be utilized or what is the maximum length of breeding (e.g., 3 breeding cycles or 1 year)?

## 5. Final Disposition

What is the final disposition of these adults at the conclusion of their breeding program?

## 6. Offspring

How many offspring are anticipated from each breeding or time-pregnancy?

## 7. Final Disposition

What is the final disposition of any offspring not utilized in the experimental program (e.g., euthanasia, replacement of retired breeders, transferred to another protocol)?

## 8. Genotype

Describe the sample collection method used for genotyping animals, including age at time of genotyping. Include tissue sampled in Appendix D.

## 9. Phenotype

Will any offspring have any known or anticipated clinical health concerns (immunocompromised, severe diabetes, ataxia, prone to dermatitis, etc. see also #9.c.)?

*Guide for the Care and Use of Laboratory Animals: Eighth Edition
http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf



TABLE 3.2 Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups*

| Animals | Weight, g | Floor Area/Animal,[a] in.$^2$ (cm$^2$) | Height,[b] in. (cm) | Comments |
|---|---|---|---|---|
| Mice in groups[c] | <10 | 6 (38.7) | 5 (12.7) | Larger animals may require more space to meet the performance standards. |
| | Up to 15 | 8 (51.6) | 5 (12.7) | |
| | Up to 25 | 12 (77.4) | 5 (12.7) | |
| | >25 | ≥15 (≥96.7) | 5 (12.7) | |
| Female + litter | | 51 (330) (recommended space for the housing group) | 5 (12.7) | Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d] |
| Rats in groups[c] | <100 | 17 (109.6) | 7 (17.8) | Larger animals may require more space to meet the performance standards. |
| | Up to 200 | 23 (148.35) | 7 (17.8) | |
| | Up to 300 | 29 (187.05) | 7 (17.8) | |
| | Up to 400 | 40 (258.0) | 7 (17.8) | |
| | Up to 500 | 60 (387.0) | 7 (17.8) | |
| | >500 | ≥70 (≥451.5) | 7 (17.8) | |
| Female + litter | | 124 (800) (recommended space for the housing group) | 7 (17.8) | Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d] |

| Appendix C | Surgery & Management of Surgical Pain and Distress |
|---|---|

1. **Complete description of surgical procedures** – List details for each surgical approach noted in question #16.

**Surgical site preparation**

The surgical site is shaved and prepared using povidone scrubs, followed by alcohol and povidone solution, while the surgeons prep ten minutes with betadine scrub or other agents upon consultation with the CCR. Surgeons, fully gowned, standing and wearing gloves, drape the animal using aseptic technique.

**Surgical approach**

Surgical and Anesthetic Protocol

The monkey is fasted for 12-24 hours prior to surgery but allowed ad lib water. The CCR staff will administrate ketamine + Atropine for induction and then move the animals to the prep area. Isoflurane is administered via face mask (typically 2-4%) to increase relaxation & facilitate intubation. After intubation, the animal is maintained with isoflurane (typically 1-3%) delivered via oxygen carrier. The monkey is transported from its home cage to the surgical suite ([redacted]) on a cart draped with a sheet. The CCR staff will prepare the monkey for the surgery. Heart rate, respiratory rate and temperature are monitored.

Surgical Procedures

***For behavioral experiments that need to measure eye position in high resolutions*** with the search coil technique, a head connector holder and an eye coil in each eye will be implanted (Surgery: head connector holder; Eye coil; described below). Eye coil is to be implanted in one eye after about 8 weeks of recovery from the head connector holder surgery. Eye coil may be implanted in the other eye after 6 weeks of recovery..

***For neurophysiological experiments,*** a recording chamber will be implanted stereotaxically on the skull (aimed at the 3$^{rd}$ or 6$^{th}$ cranial nucleus, the vestibular nuclei, the cerebellar flocculus or cortical eye fields, etc) to permit entry of microelectrodes (Surgery: Recording well described below). In each monkey, a second recording well may be implanted so that recordings can be obtained from the bilateral structures.

***For experiments that need to measure the force of the extraocular muscles***, muscle force sensors will be implanted on the medial and/or lateral rectus muscles of one eye (Surgery: Muscle force sensor).

***Head connector holder Implant***

The head connector holder is a small stainless steel cylinder attached to four short plates, each has 2-3 screw holes on it. It must be rigidly attached to the skull so eye coil or muscle force transducer wire leads can be attached to the mini-connectors

secured to it by dental acrylic. A single midline incision is made over the location where the connector holder is to be attached. The skin is reflected back, and the wound margins protected with gauze. Periosteum and muscle tissue are blunt dissected and reflected away from the bone, the short plates of the connector holder are attached to the skull using surgical grade titanium or stainless steel bone screws. The screws are hand tapped into the bone but do not penetrate the dura. After the plates are attached to the skull, the skin and subcutaneous tissue are closed completely over each plate with sutures. In contrast to the previous head holder implant, the connector holder implant reduces open wound margins to minimal size, which reduces infection and permits easy cleaning of the implant. Most importantly, this head implant will not be used to restrain the monkey's head and therefor, will not be subjected to stress and get loose over the years.

***Eye coil implant***

An eye coil or ring, consisting of sterilized metal wire, is implanted beneath the conjunctiva of an eye. The conjunctiva is opened about the limbus, blunt dissected from the sclera, and reflected back. The eye coil or ring is placed on the eye beneath the reflected conjunctiva. The coil/ring is attached to the eye with either 6-0 or 8-0 mersilene. We have used this technique over the past 20 years successfully. At the temporal margin of the eye, the ends of the coil wire are led subcutaneously from the orbit using a surgical needle to guide the wire. A small (5 mm) skin incision is made dorsolateral to the outer canthus of the eye, and the wire from the orbit is brought out from this incision. The conjunctiva is replaced over the coil, and may be drawn together with 2 or 3 mattress stitches of 4-0 absorbable vicryl. The exposed eye coil leads are led subcutaneously to the skull and brought out at the base of the head holder implant (see below) where they are attached to a connecter mounted on the implant. The incision at the outer canthus is closed with 1 or 2 dermalon (3-0) interrupted sutures.

***Recording chamber***

The recording chamber (ca 22 mm diameter cylinder) must be aimed stereotaxically at the desired recording site in the brainstem, cerebellum or cortex. A scalp incision is made at the implant site. The skin is reflected back, and the wound margins protected with gauze. Periosteum and tissue are removed completely from the bone. After the bone is exposed, a 5-20 mm diameter craniotomy is made using a trephine at the stereotaxically determined site. Extreme care is taken not to puncture or scratch the dura. Bone bleeding is controlled with bone wax if necessary. Bone screws (~6) are attached to the skull. The recording well is then placed above the craniotomy and between the screws, and lowered until its base just contacts the dura or the bone. It is attached to bone screws with dental acrylic and sealed using a cap. For recording, the cap may be removed, providing access to the brain through the well. The cap is designed so that the monkeys are unable to remove it in their cages.

***Muscle force sensors***

Muscle force sensors are placed over the rectus muscle. The conjunctiva is reflected as for the eye coil surgery. Using a muscle hook, the distal tendon of the lateral or medial rectus muscle is exposed and gently pulled away from the globe. The sensor is

slid over the muscleand tied down with 8-0 mersilene. The lead wires (identical to eye coil wire) are brought out of the orbit in the same fashion as the eye coil wire and attached to a connector on the head implant. Two sensors and an eye coil may be implanted together in an eye in a single surgery.

***Inactivation Procedure***

Monkeys trained and prepared with recording chambers and eye coils/eye tracker will be used to locate and record from the brainstem region containing cells whose activity correlates with active gaze stabilization. Once the region has been well characterized, an injectrode or a 1ul Hamilton syringe will be advanced through the recoding chamber to the area of interest to make a pressure injection. An injectrode is assembled by inserting a thin recording electrode into a sharpened narrow (32G) cannula suitable for injections. A GABA agonist, Muscimol (up to 1.5ul of 1% Muscimol in saline) will be injected into the area of interest, while the animal performs the oculomotor tasks outlined in the protocol. We will then record any changes in gaze behavior. Muscimol has a fairly long period of effectiveness (3-6 hours). We will monitor the condition of the animal until standard saccadic eye movements are seen. Since normal ocular activity reappears, one can make subsequent muscimol injections on subsequent recording days. The target area is in the pons and rostral medulla, is far from respiratory and blood pressure control areas in the caudal medulla that could have negative effects on the animal health.

***Tracer procedure***

The neuroanatomical component of the study will take place once the recording and inactivation experiments have been completed. It will be the e last experiment for a monkey. The recording chamber will be used to make the injection. An injectrode or 1 ul Hamilton syringe will be advanced through the recoding chamber to the area of interest to make a pressure injection. One of the following tracers will be injected into the area containing the active gaze stabilization neurons: [0.02 ul of 2.0% wheat germ agglutin conjugated to horseradish peroxidase (WGA-HRP), or 0.2 ul of 4.0 % Biocytin, or 0.2 ul of 10 % biotinylated dextran amine (BDA)]. In the case of WGA-HRP or Biocytin, the animal will survive for 1-2 days to allow the tracer to transport. In the case of the BDA, the animal will survive for up to 21 days to allow the tracer to transport. At that time, the animal will be sacrificed by overdose with sodium pentobarbital (50 mg/kg, IV) followed by cardiac exsanguination with sequential buffered saline and mixed aldehyde fixatives and decapitation to allow removal of the brain.

***Euthanasia Procedures***

A deep surgical plane of anesthesia will be achieved by ketamine induction (10 mg/kg, IM) followed by sodium pentobarbital, 50mmg/kg, IV until loss of corneal and withdrawal reflexes. After the surgical plane of anesthesia is attained, the animal is euthanized by transcardiac infusion of fixative (formalin). Brain tissue will be collected to perform histology for locating recording sites. This also ensures the death of the terminated animals.

**Wound closure method, materials, and removal plan**

The incisions are closed with 1 or 2 dermalon (3-0) interrupted sutures. The incisions are cleaned during recovery. Sutures are removed 7-10 days later. The CCR staff will be consulted on improving these procedures.

## 2. Provide a complete formulary of medications related to surgical procedures:

| | Agent | Dose | Route | Frequency/Duration | Pharmaceutical Grade |
|---|---|---|---|---|---|
| Pre-anesthetic | Atropine<br>Ketamine | 0.05 mg/kg<br>10-20 mg/kg | IM<br>IM | One time<br>One time | ☒Yes ☐No |
| Pre-operative analgesics | **Carprofen**<br><br>**Buprenorphine SR**<br><br>**Or other drugs at the discretion of the CCR staff** | **4mg/kg**<br><br>**0.045mg/kg** | **SC**<br><br>**SC** | **One time**<br><br>**One time** | ☒Yes ☐No |
| Post-operative analgesics | **Carprofen**<br><br>**Ibuprofen**<br><br>**Or other drugs at the discretion of the CCR staff** | **4mg/kg**<br><br>10 mg/kg | **PO**<br><br>**PO** | **every 24 hours as needed**<br><br>BID 3-5 days | ☒Yes ☐No |
| Anesthetics | **Isoflurane** | **1-2%** | **inhaled** | **continuous** | ☒Yes ☐No |
| Fluid/blood replacement | Saline | | | | ☒Yes ☐No |
| Antibiotics and topical steroids | Upon CCR consultation | | | **Upon CCR consultation** | ☒Yes ☐No |

For non-pharmaceutical-grade compounds:

**a.** **Justify** the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

**b.** Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

## 3. Anesthesia

a. Who will conduct the anesthesia procedure(s)?

| |
|---|
| CCR staff |

b. Describe experience and training with anesthesia.

c. What criteria will be used to assess anesthetic depth and how will this be monitored?

| |
|---|
| Anesthesia will be administered and monitored by CCR staff. In general, absence of withdrawal and corneal reflexes, and stable cardiovascular responses will be used as criteria. |

## 4. Aseptic Technique

a. What procedures will the surgeon use to prepare himself/herself for aseptic surgery?

| |
|---|
| • Open brush<br>• Turn tap on<br>• Wet hands and arms under running water<br>• Apply anti-microbial solution to hands and arms<br>• Remove pick from brush packet and clean nails<br>• Scrub all sides of each digit, the back of the hands and palms<br>• Rinse off solution from fingertips to elbow<br>• Allow excess water to drain from the elbows into the sink<br>• Walk to gowning and gloving area with hands held away from the body keeping hands higher than elbows. |

b. How will the instruments be prepared for aseptic surgery? (Sterile instruments must be used for each animal.)

| |
|---|
| Gas/autoclave sterilization |

## 5. Location of Procedures

Where will the surgical procedures be conducted?

| |
|---|
| [redacted] |

## 6. Post-procedural Care

a. Who will conduct and document post-procedural animal care (post-op analgesia, nursing care, etc.)? *Documentation will be checked at IACUC semi-annual inspection.*

| |
|---|
| [redacted] |

b. Include a plan of monitoring frequency, duration and intervals of post-op analgesia, nursing care, etc.

| |
|---|
| **Recovery**<br>At the end of a surgical procedure isoflurane is reduced gradually and oxygen is given to wake up the monkey. As the monkey begins to swallow, |

the deflated endotracheal tube is removed and the monkey may be returned to home cage for recovery.

**Post-op care**

*Head implant surgery (connector holder and recording chamber)*: the incisions are cleaned during recovery and Ibuprofen (10 mg/kg), Buprenorphine SR (0.045mg/kg IM) or Carprofen (4mg/kg) may be administered upon consultation of the CCR staff. Sutures are removed 7-10 days later if needed.

*Eye coil or muscle force sensor surgery*: Because there is occasional inflammation, topical steroids and antibiotics (upon consultation of the CCR staff and ophthalmologists) will be administered pre-op and continued post-op if needed. Ibuprofen (10 mg/kg PO BID 3-5 days), Buprenorphine (0.045 mg/kg SC)or Carprofen (4mg/kg) or other drugs at the discretion of the CCR staff will be provided as needed.

**Assessment of health status of animals with any form of exteriorized head implant**

The lab staff will work closely with the CCR staff to monitor the health status of animals with recording chambers and the head connector holder. The lab staff will inspect the conditions of the head implants and document their weights, water/food intake (if water is scheduled), general behavior and conditions of the head implants. Whenever we find indications concerning monkeys' health, the CCR staff will be consulted and if necessary a treatment plan will be developed. The lab staff will work closely with the CCR staff to implement this plan to ensure effective treatment.

**Regular recording chamber maintenance**

The following procedures developed by the CCR based on the APV recommendations are adopted as our standard protocol. Upon consultation of the CCR staff, cleaning/maintenance of the recording chamber will use rotations of several disinfect solutions, such as chlorhexidiene, betadine, dakin's solution or other alternatives indicated by the CCR.

Clean skin margins by removing any exudate if found around recording chamber using cotton swabs saturated with a disinfect solution. If infection is confirmed, upon consultation of the CCR, appropriate antibiotic ointment will be applied to skin margins.
Remove chamber cap and aspirate material from the chamber.
Rinse the chamber multiple times by filling with a disinfect solution.
Using sterile cotton swabs saturated with a disinfect solution thoroughly clean the interior surface of the chamber.
Rinse the chamber again by filling with a disinfect solution followed by aspiration to remove any remaining material.

Using sterile cotton swab clean the exterior surface of the chamber with a disinfect solution.
Apply antibiotics into the recording chamber as instructed by the CCR.
Put back the cap on recording chamber.

**Deep clean of head implants with signs of infection**

Prior to any procedure, examine the monkey for any signs of infection- exudate, pus, or swollen skin margins. Contact the CCR staff by calling [redacted] if any of these are observed.

If infection is confirmed, the following procedures will be used to treat infection under CCR supervision.

Before sedating monkeys with ketamine, ensure animal has been fasted for at least 8 hours.

Cleaning is done in the appropriate area after drug takes effect.

If there is any hair under and around the recording chamber. Remove them with clippers, razors, and small scissors. After hair removal, examine the skin underneath the implants for any signs of infection that may have been covered by the hair. Contact the veterinary staff by calling 4-1385 if any signs of infection are observed.

Thoroughly scrub all infected skin margins around head implants with 4X4 gauze and long “Q-tips” with the agents instructed by the CCR. Care should be taken to avoid getting scrub into the monkey eyes. After scrubbing, remove the scrub with sterile water, peroxide, saline, alcohol or agents instructed by the CCR.

After removing all scrub, coat the infected skin margins with antibiotic ointment as a protective antimicrobial barrier (upon consultation with the CCR staff).

Finally, clean and rinse the work area and sink after use. The exudate and blood from the monkeys are all biohazards and should be appropriately decontaminated. Scissors, clippers, and razors should also be thoroughly disinfected.

c. What is the expected time from end of procedure until animal(s) are returned to home environment?

1 hour

## 7. Emergency Contacts

Provide emergency contact information (pager/phone number) for evenings or weekends concerning post-operative complications.

[redacted]: cell [redacted], phone [redacted]
[redacted] cell [redacted], phone/[redacted]

## Appendix D Collection of Biological Samples from the Live Animal

Biological samples include blood collection, urine collection, ascites, tail tips for DNA, cerebrospinal fluid, biopsy, etc. Appendix D is completed for all sample collections from live animals, including under terminal anesthesia. Appendix D is not required for samples taken after euthanasia.

1. Indicate the body fluid or material to be collected.

2. Indicate the method and site of collection.

3. Indicate the volume of fluid or amount of material to be collected.

4. Indicate the frequency of collection.

5. Will the animal(s) be anesthetized or sedated during this procedure?
☐No ☐Yes

If No, describe restraint method. (Note: If methods require a prolonged period of restraint, Appendix G is required.)

If Yes, list agents used for anesthesia and anaglesia:

| Agent | Dose | Route | Frequency/Duration | Pharmaceutical Grade |
|---|---|---|---|---|
| | | | | ☐Yes ☐No |
| | | | | ☐Yes ☐No |
| | | | | ☐Yes ☐No |
| | | | | ☐Yes ☐No |

For non-pharmaceutical-grade compounds:

**c.** **Justify** the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

**d.** Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

## Appendix E Antibody Formation /Hybridoma & Ascites

1. Indicate what antigen will be used: Click here to enter text.

2. Indicate what vehicle/adjuvant will be used: Click here to enter text.

   a. Initial immunization: Click here to enter text.
   b. Subsequent immunizations: Click here to enter text.
   c. Anticipated complications/side effects: Click here to enter text.

3. Indicate sites for immunization: Click here to enter text.
4. Describe skin or animal preparation prior to injection: Click here to enter text.
5. Indicate route of administration: Click here to enter text.
6. What is the total and per site injection volume? Click here to enter text.
7. What is the frequency/duration of immunization (e.g., 1 injection every 2 weeks for 3 injections)? Click here to enter text.

**ASCITES PRODUCTION**

Fluid accumulation associated with ascites/hybridomas should not become greater than 10% of body weight. Animals should be euthanized if they become moribund.

8. Indicate the maximum volume of ascites fluid to be collected per sampling (ml/mouse) and the method of collection (skin prep, gauge needed, gravity vs. suction, etc.)

9. Indicate the number of fluid collections and anticipated frequency of collection.

10. Describe procedures used to care for and monitor the health of animals with ascites and the point of euthanasia.

Consult: http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf

http://oacu.od.nih.gov/ARAC/documents/Ascites.pdf

## Appendix F Administration of Drugs/Test Compounds

All agents given to the animals **must** be listed in this section with the exception of veterinary pharmaceuticals (antibiotics for treatment, anesthetics, and analgesics for treatment). Those will be listed in Appendix C.

NOTE: A pharmaceutical-grade compound (PGC) is defined as any active or inactive drug, biologic or reagent, for which a chemical purity standard has been established by a recognized national or regional pharmacopeia (e.g., the U.S. Pharmacopeia (USP), British Pharmacopeia (BP), National Formulary (NF), European Pharmacopoeia (EP), Japanese Pharmacopeia (JP), etc.). These standards are used by manufacturers to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy.[1]
The Food and Drug Administration (FDA) maintains a database listing of FDA approved commercial formulations for both FDA approved human drugs (the Orange Book) and veterinary drugs (the Green Book).

Provide the following information:

| Agent | Dose | Volume | Vehicle | Route | Frequency | NDC or CAS# | Hazard? | Pharmaceutical Grade |
|---|---|---|---|---|---|---|---|---|
| biocytin | 4% | 0.2 ul | saline | Intra-cerebral | once | **98930-70-2** | **No** | ☐Yes ☒No |
| biotinylated dextran amine (BDA) | 10% | 0.2 ul | dH2O | Intra-cerebral | once | **Not Found on MSDS** | **Nonhazardous, Wash off skin, and out of eyes** | ☐Yes ☒No |
| WGA conjugated horseradish perox (WGA-HRP) | 1-2% | 0.02ul | dH2O | Intra-cerebral | once | **Not Found on MSDS** | **Irritant, Harmful if swallowed or inhaled** | ☐Yes ☒No |
| Muscimol | 1 % | 1.5ul | saline | Intra-cerebral | Once/ session | **2763-96-4** | **Yes** | ☐Yes ☒No |

NDC# is preferred over CAS#, if available. The NDC# will be on the bottle or box if the substance is a pharmaceutical. If there is no NDC# then include the CAS#. CAS# and hazard information can be obtained from the MSDS sheet through the UMMC Intranet (http://www.umc.edu/intranet/index.php). Choose the "MSDS On-Line" link under "Hot Spots".

1. Describe any potential adverse side effects that may result in the animal from the administration of this material. If agents are unknown or their potential side effects are not documented, provide a reasonable estimate of the effects of the general class of chemicals (e.g., compound may have sedative properties, compound will likely produce diarrhea, etc.).

Biocytin, biotinylated dextran amine (BDA) and wheat germ agglutinin conjugated to horseradish peroxidase (HRP) have been used in many other laboratories with the methods and amounts described for more than 15 years with no sign of ill effects on the animals or necrosis at the injection site at time of autopsy.

Muscimol has been used for CNS inactivation since 1985. Muscimol must be injected with some care, since it inactivates the region around the injection site. We will be injecting in the rostral medulla far from respiratory and cardiovascular sites. We will only inject this substance once we have characterized the physiology of the area, and found it to be related to gaze changes involving the eyes or head. We will start with small injections, and look for the lowest dosage that produces changes in gaze behavior. When injected in this way, Muscimol only produces specific effects on the movements of the eye. For example, its eye movements to targets will be slower.

2. For each hazardous material, a Hazard Use form must be completed and attached it to the protocol. How many Hazard Use forms are included?

None of these compounds will be excreted or available on the surface of the animal, so no special procedures are required for their administration. Routine lab procedures, use of gloves, avoiding spills, etc. are all that are required when handling them in the solution. When weighing it out and placing them in solution, however, respiratory protection (N95 or equivalent) is required in the solution preparation area to prevent potential airborne exposures. Personal protection equipment including goggles, gloves will be required. Wash any contaminated surfaces with a soap and water solution, and follow waste disposal procedures (dispose of the hazardous chemical waste through EHS) for eliminating or sufficiently reducing the exposure).

<link to Hazard Use form>

3. For non-pharmaceutical-grade compounds:

   a. **Justify** the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

Neuroanatomical tracers and muscimol are necessary for these experiments. None of the test compounds are supplied as pharmaceutical grade as they have no clinical uses. Therefore, in order to carry out the experiments as designed, it is necessary to utilize non-pharmaceutical grade test compounds. The neuroanatomical tracers have been used in neuroscience experiments since the 1970s with no reports of them causing infection or other health hazard to the animal or investigator. There are no health effects in the quantities being used in these experiments. Muscimol has been used by neurophysiologists since 1985. There are no permanent effects if used in the appropriate amounts and applied to none-critical areas of the brain.

   b. Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity,

osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

Animal health and welfare are ensured by measures listed below:
(1) injection the smallest volume needed to produce adequate compound transport or inactivation.
(2) formulating the test compounds in non-reactive vehicle (distilled H2O or saline) in disposable storage vials that are then stored in the refrigerator.
(3) ensuring the injection syringe is sanitized prior to injection of test compound.
(4) these are biological compounds that cannot be sterilized if they are to maintain the biological activity necessary for the study to be successful.

Reference: UMMC Chemical Safety Manual
http://ehs.umc.edu/documents/ChemicalSafetyPolicy2010.pdf

Please remember that the use of any hazardous material in animal rooms requires that a sign be posted in that room and on the cages containing the hazard in accordance with the policy on Signage for Hazardous Studies.

[1] AAALAC Frequently asked questions about Non-Pharmaceutical Grade Compounds

## Appendix G Prolonged Physical Restraint

Physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation. Examples of prolonged physical restraint include: chairing of nonhuman primates, chronic harness restraint of metabolic animals, and tube restraints for rodents. For additional information, consult the IACUC's policy statement on Prolonged Physical Restraint.

1. **Justify** the need for prolonged physical restraint.

To prevent monkeys grab people, monkeys will be seated inside a primate chair.
To accurately measure eye movements using the eye tracker or the search coil technique, monkeys' head needs to be supported in a natural upright position inside of the apparatus.

2. Describe the restraint device.

**The monkey chair**

As shown in the right, the chair is designed to be opened from the back and the monkey is guided into the chair using the pole. The chair-back can then be closed and the pole removed. The primate chair needs to allow monkeys with various sizes to sit comfortably. We will consult the CCR staff to evaluate whether a monkey is too big for the chair. For oversized monkeys, we will modify the existing chairs or make new chairs.

**Head position support**

The monkey's head is held in a natural upright position supported by a face mask made from Plexiglas and padded with moleskin that fits snugly across the snout and a u-shaped neck/head collar piece positioned to the back of the head to prevent withdrawal from the face mask. Different from traditional head restraining devices, the mask allows small head movements while the head is being snuggly supported. Each monkey will have its own pads, which will be replaced if needed.

3. Describe the details of how the animal(s) will be adapted to the restraint device.

**The primate chair**

The CCR has been consulted on using positive reinforcements to train the monkeys get out of the cage quietly and sit in the chair with minimal efforts. Each monkey is fitted with a collar that can be hooked to a pole with a clamping hooked end. The CCR staff will be consulted on choosing collar materials (nylon, plastic, etc). Using fruits as rewards, monkeys are trained first to come to the front of the cage, then out of the cage on the floor, then being guided to the monkey chair that fits its size. The chair is designed to be opened from the back and the monkey is guided into the chair using the pole. The chair-back can then be closed and the pole removed. The monkey will be given additional rewards in the chair. The training procedure will be repeated a few times a week. We will gradually increase the duration of monkeys sitting in the chair, from 30 minutes to 4 hours over a few weeks. A monkey is considered well trained when it will leave their home cage quietly and climb into the monkey chair with minimal effort. The monkey is returned to the home cage in a similar fashion. While this process will on average take about 8 weeks, monkeys are expected to exhibit individual differences. We will train each monkey on its own schedule. Its progress will be defined with milestones for advancement. For example, the first milestone is to have the monkey come to the front of the cage and present its collar. Once the monkey achieves this milestone, it will be trained to get out of the cage quietly and get into the chair.

**Head position support**

For behavioral training and testing, the monkey will be seated in the primate chair, which will be secured in the vestibular stimulator in the lab that can provide whole-body rotation,

translation or tilt. The monkey's head is held in a natural upright position supported by a face mask made from Plexiglas and padded with moleskin that fits snugly across the snout and a u-shaped neck/head collar piece positioned to the back of the head to prevent withdrawal from the face mask. The mask is attached to the apparatus and can be rotated in the horizontal direction if needed. Therefore, the monkey can be allowed to rotate its head in the horizontal direction with the mask. Different from traditional head restraining devices, the mask allows small head movements while the head is being snuggly supported. Since the face mask supports the monkey's head in a natural upright position, it does not resist the setup. The monkey will move its head into the mask while drinking juice from the lab staff.

After the mask is put on the monkey, it will be given juice when looking at a visual target. Based on our experience, the monkey can sit in the chair and do the task for several thousand trials, which last up to four hours. The monkey also can take a break from the task or even take a brief nap, as indicated by the slow drifting of the eyes. We will gradually increase the duration of monkeys sitting in the chair, from 30 minutes to 4 hours over a few weeks.

4. a. What is the duration of a restraint period?

2-4 hours

b. How frequently will an animal receive the restraint (e.g., daily, once per week, every month)?

Monday-Friday, up to 5 times per week.

5. Are animals monitored during the restraint period? ☐No ☐Yes
How often?

All the time.

6. Are there any anticipated problems as a result of the restraint device (e.g., skin lesion from harness, moist dermatitis, etc.)?

Since monkeys are trained to voluntarily accepting the pole, the chair and the mask, we do not expect problems of adapting to these devices. If any lesion occurs during training and testing, the lab staff will consult the CCR for treatment and solutions to prevent it from happening again.

## Appendix H Multiple Survival Surgical Procedures

A major surgical procedure is defined as a surgical intervention that penetrates or exposes a body cavity (peritoneal, thoracic, cranium), produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Guide, 2011). Multiple procedures are those whereby an animal will regain consciousness after each procedure. Procedures must be described in Appendix C. A surgery followed by a second procedure where the animal is euthanized is not considered multiple surgical procedures.

Surgeries performed on the animal prior to the animal's arrival at UMMC (e.g., ovariectomy procedure performed by vendor) must be considered. For additional information consult the IACUC's policy statement on Multiple Major Surgical Procedures.

1. **Justify** the need for multiple major surgical events in a single animal.

To record neuronal activities, eye movement at high resolutions and muscle force, recording chambers, eye coils/head connector holder, muscle force transducers will be needed. As long as a year may pass from the first to the last surgery. This sequence of separate surgeries is dictated by concern for the animals' wellbeing and for scientific reasons. If all the implants were made at once, the time required for surgery could exceed 12 hours. The initial surgery is needed to train the animal. If the recording well(s) were implanted, they could not be used for at least a year and the dura exposed in the well would become thickened requiring surgical scraping and inviting possible infection. In consultation with the CCR staff, it is decided that it is best to serially perform shorter procedures timed to instrument the animal as close as possible temporally to the time of experimental recording. These procedures are standard practices for this type of experimental protocol and produce good results with a minimum of discomfort and stress for the animal. If implants (eye coils, recording chamber, or head connector holders, etc) become dislodged/damaged/non-functional, they will be replaced using the procedures described in Appendix C. The CCR staff will be consulted on whether the head implant needs to be repaired.

2. What is the time interval between the surgical events?

Monkeys are allowed 8 weeks to recovery from a head connector holder surgery and 6 weeks to recover from an eye coil surgery and a muscle force transducer surgery.

## Appendix I Food and/or Fluid Regulation

The Guide (2011) states: "Regulation of food or fluid intake may be required for the conduct of some… research protocols. The regulation process may entail ***scheduled access*** to food or fluid sources, so animal consumes as much as desired at regular intervals, or ***restriction***, in which the volume of food or fluid consumed is strictly monitored and controlled." The least restriction necessary to achieve scientific objectives while maintaining animal well-being should be used. For additional information consult the IACUC's policy statement on Food and/or Fluid Regulation.

1. Will ☐FOOD or ☒FLUIDS be ☒**scheduled** or ☐**restricted**?

**Justify** the need to schedule or restrict food and/or fluid.

**In the period when animals are taken to the lab for testing**, they will not have automatic water in the home cage. Instead, they will be trained to get fluids in the lab by looking at visual targets presented on a screen or TV. The adjustment in water schedule is to encourage monkey participation and get used to drinking liquid in the lab, where they will be offered adequate amount of liquid to stay hydrated for a day. Since the tasks are natural

to monkeys, they are allowed to drink as much liquid as desired by simply looking at the target. Indeed, monkeys usually get ~300ml juice in the lab, which is adequate for them to stay hydrated (~30ml/kg for a 10kg monkey, or 2400ml/day for a 80kg human). We also make sure monkeys will get additional 50ml in the home cage.. While we do not have an upper limit of fluid intake, we do make sure the monkey has at least 30ml/kg in the lab. If monkeys have automatic water in the home cage, they may not be interested in getting fluid in the lab. If monkeys are not motivated to do the tasks, we will not be able to get consistent behavioral assessment of their vestibular and oculomotor systems.

Over the past 25 years, we and other labs worldwide have been using this water scheduling method. Monkeys not only do well in these tasks, but also are maintaining good conditions. To ensure monkeys in good health, the lab staff [redacted]) will maintain records of fluid consumption and body weights. If a monkey is found to have a loss of body weight over 10%, we will consult the CCR immediately to determine whether it needs to have access to automatic water.

2. Check all methods that will be used to ensure adequate nutritional intake and hydration.

| METHOD | | FREQENCY OF CHECKS |
|---|---|---|
| Body weight | ☒ | Weekly if on scheduled water |
| Urine output | ☒ | Monday-Friday if on scheduled water |
| Fecal output | ☒ | Monkday-Friday if on scheduled wate |
| BUN | ☐ | |
| Hct | ☐ | |
| Food intake | ☒ | Weekday if on scheduled water |
| Other | ☐ | |

3. Restriction protocols typically base the restriction amount relative to a baseline, (free-choice consumption) parameter (body weight, intake amount). What will this restriction amount use as the baseline?
Free access water
What is the maximum restriction for any animal?

~30ml/kg

4. Growing animals must be frequently re-assessed to ensure normal growth patterns. If not using mature animals, what provisions will be made for these animals to assure that their nutritional needs are maintained?

Growing monkeys of 3-5kg will be acquired initially. After years in the lab, these monkeys become mature and grow to ~12kg. Monkey liquid/food intake and body weights will be monitored and the status of monkeys' health will be evaluated by the CCR and the lab staff. When there are concerns on body weight or growth pattern, the CCR will be consulted to develop a plan to compensate.

5. Describe the protocol for regulating food and/or water intake.

**In the period when animals are not taken to the lab for testing**, they will have automatic

water in the home cage on weekdays and weekends.
**In the period when animals are taken to the lab for testing**, they will not have automatic water in the home cage. The water schedule is adjusted during the training so that monkeys get used to drinking water primarily in the laboratory by simply looking at visual targets presented on a screen or TV in front of them. In a typical trial, a monkey is required to maintain fixation on a visual target for about 0.5-1 s to obtain 2 drops of fruit juice (~0.2ml). Monkeys usually finish about 2 to 3 thousand trials within 2~4 hours and drink ~300ml juice, which is adequate for them to stay hydrated. In days when fluid intake is less than 30 ml/kg in the lab, the monkey will be given the balance amount of fluids continuously without any requirements. Additional 50ml liquid will also be provided in the home page to help monkeys eating food. On Fridays, lab staff will provide a full bottle of 1000ml water in the home cage. On Saturdays, Sundays and holidays, the CCR staff will provide a full bottle of 1000ml water or reconnect the water line.

6. How long will animals be on the regulation protocol?

Monday to Friday during behavioral testing period.

7. Will animals have any access to unrestricted food or water at any time?

Yes. Water is unrestricted all the time when not in the training/testing phase.. The study does not need to regulate food. Upon consultation with the CCR, each monkey will be given adequate food for their age and body weight.

8. Who will be responsible for administering and documenting the regulation?

[redacted]

**Note: NPO procedures for pre-surgical fasting are not included in this consideration. NPO procedures shall not extend for greater than 24 hours; if surgical delays are encountered, the animals should be fed and re-fasted prior to the next scheduled procedure.**

## Appendix J Animal Pain and/or Distress

The management of post-procedural pain or distress is typically addressed with the use of appropriate pharmacologic and non-pharmacologic methods (see Appendix C). Appendix J should be completed if there are any procedures that are proposed that may cause more than momentary, slight pain or distress during which the appropriate sedatives, analgesics, or anesthetics will be **withheld** or in **which chronic pain or distress is induced**. Proposals which incorporate animal manipulations or procedures which may create more than momentary pain and distress (noxious injections, tumor growth, sequelae to compound administration, etc.) should also be addressed. For additional information consult the IACUC's policy on Animal Pain and/or Distress.

1. **Justify** the scientific need to withhold appropriate drugs or induce the pain/distress.

2. What is the duration of time that an animal may experience this pain/distress?

3. Describe non-pharmaceutical means to alleviate pain/distress (soft bedding, social housing, supplemental heat, etc.).

4. Describe situations where an animal may be removed prematurely from a study.

5. Describe those procedures whereby animals are likely to experience more than momentary pain or distress as a result of manipulations or procedures (noxious injections, tumor growth, sequelae to compound administration, etc.).

6. Will any anesthetics, analgesics, or tranquilizing drugs be used to reduce this pain or distress?

## Appendix K Progress Report

1. Give a brief description of the work performed on these projects in the past 3 years. If progress did not occur or was less than expected, please give a brief explanation.

In the past three years, we have conducted experiments to investigate the neural mechanisms underlying gaze stabilization during active head rotation and processing of global and local features. We made progress in obtaining new data for publication and grant applications.

2. List any publications, abstracts, and/or presentations coming directly from the work performed on these projects in the past 3 years.

[redacted] Rapid processing of a global feature in the visual ON pathways of behaving monkeys. Front. Neurosci. 11:474. doi: 10.3389/fnins.2017.00474.

[redacted] Modulation of effects of pre-cues on saccade latency by spatial and contextual factors in behaving monkeys, SFN, 2018.

[redacted]. Oculomotor plant hypothesis (OPH) revisited: Abducens neuron behaviors during combined eye-head gaze shifts, disjunctive smooth pursuit and sleep in monkeys. SFN, 2019.

3. Answer the following questions in regard to the last year of the previous version of this protocol.

## I. Animals

1. Have any unanticipated (morbidity, mortality, inability to collect data) events occurred in the past year?
   ☐ Yes ☒ No
2. Has any mortality occurred prior to the anticipated end-point of an experiment or as a result of surgical manipulation?
   ☐ Yes ☒ No
3. Have any animals been euthanized prior to the anticipated end-point of an experiment?
   ☐ Yes ☒ No
4. Did any animals show signs of morbidity or sickness following experimental manipulation other than what was detailed in the protocol?
   ☐ Yes ☒ No

**If yes to 1 -4, answer #5.**

5. Describe any unanticipated events (morbidity, mortality, inability to collect data) and any identified contributing factors (e.g., recurring postoperative complications, excessive or unanticipated mortality rate, unplanned event that causes the removal of an animal(s) from an experiment for a period of time, loss of implant, etc.).

**If the protocol involves breeding:**

**Breeding:** Animals born over the past year as part of this protocol

| Species | Strain | # of pups born in last year | # of pups used in the last year for experiments |
|---|---|---|---|
| | | | |

What was the final disposition of any pups not used for experiments?

## II. Personnel

1. During the past year did any Occupational Health & Safety "incidents or accidents" *(needle sticks, animal bites, cuts, burns, etc.) occur that involved personnel participating* in the conduct of this study? ☐ Yes ☒ No

2. If yes, describe the event and identify any contributing factors:

3. What treatment measures were taken:

## Appendix L Behavioral Training and Testing

Useful Resources:

NIH Publication: *Methods and Welfare Considerations in Behavioral Research with Animals NIH Publication No. 02-5083, March 2002*
*http://www.nimh.nih.gov/researchfunding/animals.pdf*

American Physiological Society Publication: *Resource Book for the Design of Animal Exercise Protocols*, Feb. 2006
http://www.the-aps.org/pa/action/exercise/book.pdf

1. What form(s) of behavioral training/testing will be used?

Chair Training, head position support, oculomotor tasks.

2. Describe how the behavioral training/test is conducted (include descriptions of the devices, preliminary animal training, fluid/food restriction, reward/ positive reinforcement, duration of trial, frequency of behavioral testing, etc.).

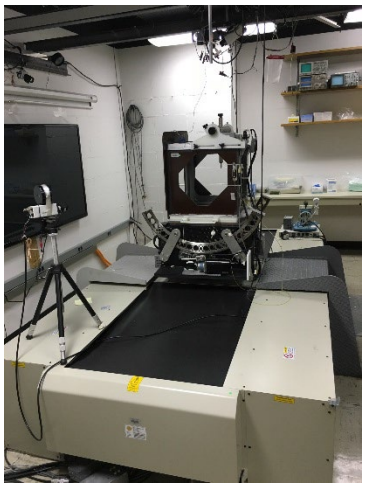
Chair Training
Each monkey is fitted with a collar that can be hooked to a pole with a clamping hooked end. The CCR staff will be consulted on choosing collar materials (nylon, plastic, etc). Using fruits as rewards, monkeys are trained first to come to the front of the cage, then out of the cage on the floor, then to the monkey chair. The chair is designed to be opened from the back and the monkey is guided into the chair using the pole. The chair-back can then be closed and the pole removed. The monkey will be given additional rewards in the chair. The training procedure will be repeated a few times a week. A monkey is considered well trained when it will leave their home cage quietly and climb into the monkey chair with minimal effort. The monkey is returned to the home cage in a similar fashion. While this process will on average take about 8 weeks, monkeys are expected to exhibit individual

differences. We will train each monkey on its own schedule. Its progress will be defined with milestones for advancement. For example, the first milestone is to have the monkey come to the front of the cage and present its collar. Once the monkey achieves this milestone, it will be trained to get out of the cage quietly and get into the chair. Monkeys need to complete this training before any surgical procedures.

Animal head position support

For recording eye movement and single unit activity during eye tracking tasks, the monkey will be seated in the chair secured in the vestibular stimulator that can provide whole-body rotation, translation or tilt. The monkey's head is held in a natural upright position supported by a face mask made from Plexiglas and padded with moleskin that fits snugly across the snout and a u-shaped neck/head collar piece positioned to the back of the head to prevent withdrawal from the face mask. The mask is attached to the apparatus and can be rotated in the horizontal direction if needed. Therefore, the monkey is allowed to rotate its head in the horizontal direction with the mask. Different from traditional head restraining devices, the mask allows small head movements while the head is being snuggly supported. Since the face mask supports the monkey head in a natural upright position, it does not resist the setup. Juice rewards will be used to train the monkey to move its head into the mask. High speed cameras can be attached to the mask apparatus to record eye positions.

Behavioral training and water/food intake assessment



After a monkey completes the chair training, it will be ready for behavioral training in the lab on weekdays. The monkey will be rewarded with juice if it fixates a stationary or moving target presented on a screen or TV in front of them while making voluntary head movement or being rotated/translated by the vestibular stimulator shown in the right photo. Monkey eye positions will be measured by the search coil technique or a video-based eye tracker. In a typical trial, the monkey is required to maintain fixation on a visual target for about 0.5-1 s to obtain 2 drops of fruit juice (~0.2ml). Monkeys usually finish about 2 to 3 thousand trials within 2~4 hours and drink ~300ml juice, which is adequate for them to stay hydrated. In days when fluid intake is less than 30 ml/kg in the lab, the monkey will be given the balance amount of fluids continuously without any requirements. Additional 50ml liquid will be provided in the home page to help monkeys eating food. The adjustment in water schedule is to encourage monkeys' participation. Records of water consumed in the laboratory, food intake in the home cage and body weights will be maintained during the testing period.

3. If an unexpected problem or event occurs in the performance of the above described behavioral training/testing procedure(s) that directly impacts the live

animal, what steps will be taken to ensure appropriate treatment is provided?

When an unexpected problem occurs, the CCR staff will be consulted to provide appropriate treatments.

4. Will animal be observed/attended throughout the duration of the trial/test?
   ☐No ☒Yes
   If No, provide rationale.

5. Describe any unique post-trial animal husbandry that may be required (e.g., dry/warm environment for animals in the Morris Water Maze, soft padding for animals on the Rod Test, etc.).

There is no unique post-trial animal husbandry.

6. List personnel involved with the actual training and indicate his/her level of knowledge as it relates to the training/testing used in the lab.

[redacted]: 14 years of working with monkeys in the lab.
[redacted]: 15 years of working with monkeys in the lab
[redacted], 5 year of working with monkeys in the lab

7. Where will the test(s) be conducted?

[redacted]

8. Will the Animal Behavior Core (ABC) be used for this testing?
   ☒ No
   ☐ Yes – Use of the ABC requires review and approval by the Core Director.

X_____
ABC Director (Paste digital copy of signature)

**Attach copies of ABC SOPs that will be used for this study.**

## Appendix N Use of Expired Medical Materials or Devices

The use of expired medical materials and/or drugs may be allowed for non-survival procedures. The attending veterinarian and the IACUC are responsible for ensuring that proposed animal activities avoid or minimize discomfort, distress, and pain to the

animal. These responsibilities cannot be met unless the veterinarian and the IACUC maintain control over the use of expired medical materials.

All anesthetics, for survival and acute procedures, analgesics, emergency drugs, and euthanasia agents must be in date.

All pharmaceuticals and medical materials (e.g. drugs, antibiotics, fluids, saline bags, disinfectant solutions, catheters, sutures, etc.) used in survival procedures must be in date.

For additional guidance see the IACUC Policy Statement *Use and Maintenance of Expired Medical Materials (Pharmaceuticals and Devices)*

1. List and describe expired medical materials and/or expired medical devices to be used and describe intended use of each item. *NOTE: All expired medical materials or devices must be clearly labeled, "Expired, for conditional use only"*.

2. Please provide a justification for the use of the expired items.

3. Describe if sterility will be required, and if so, how proper sterility will be assured.

4. Identify the room and exact location where expired items will be stored. *NOTE: Items must be kept in a separate location (cabinet, shelf, box) and must be clearly labeled, "Expired, for conditional use only"*.