Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 21-1821R
Protocol Title: Vision restoration using magnetic stimulation of visual cortex
Principal Investigator: [redacted]
Date of Action: 1/28/2021

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that DACT provide supervision for the first time a procedure is conducted. Contac[redacted]to schedule.

☒ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT [redacted], prior to starting surgeries.

☒ Other requirements: The Trocar Use SOP must be approved before work referenced in the SOP may begin.

Total # of Animals: 1
Species: NHP **Pain Category:** D

Protocol Approval Period: 1/28/2021 – 1/27/2024

Sponsor: NIH
ASU Proposal/Award #: [redacted]
Title: Psychophysical validation of visual perception generated by MEMS magnetic stimulation of primary visual cortex

Signature [redacted] Date: 2/12/2021
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

IACUC Use Only	**IACUC Protocol #:** 21-1821R
Date: 1/4/2021	☐ IBC ☐ RSC ☐ Chem

ANIMAL USE PROTOCOL
ARIZONA STATE UNIVERSITY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
(Revised May 2020)

Read "Instructions for Submitting the ASU Animal Use Protocol" before completing. **Upon approval, this protocol will become a public record so follow instructions carefully.**

PROJECT/PROGRAM TITLE: Vision restoration using magnetic stimulation of visual cortex

SPECIES REQUESTED: *Macaca mulatta*

I. PERSONNEL INFORMATION

A. A single member of the university faculty and/or Principal Investigator (PI) is considered the responsible individual.

NAME: [redacted] TITLE: Associate Professor

AFFILIATION: [redacted] Office Phone # [redacted]

Cell Phone #: [redacted] E-Mail: [redacted]

B. Additional contact, if any, for IACUC business

NAME: TITLE:

AFFILIATION: Office Phone #

Cell Phone #: E-Mail:

C. Protocol Type

☐ Non-funded research

☐ Internal Funding

Account Number:

☒ External Funding (Grant/Contract)

Granting Agency: [redacted] Deadline:

Co-Investigator(s) [redacted]

Proposal Title: Psychophysical validation of visual perception generated by MEMS magnetic stimulation of primary visual cortex

ASU Proposal or Award # [redacted]

If, ASU proposal or award number is not provided, attach a copy of the complete proposal or grant document.

☐ Teaching - Course Number and Title:

D. Protocol Status:

☒ New

☐ Renewal—Previous Protocol #:

☐ Revision—Previous Protocol #:

E. Do you plan to use Department of Animal Care & Technologies (DACT) personnel and resources? If yes, describe the support needed? (If this use is new or an expansion of previous use, contact the DACT well in advance of need). Yes. Husbandry and care, environmental enrichment, surgical assistance and pre- and post-operative care.

II. PROJECT DESCRIPTION AND PROGRAM REQUIREMENTS

The Institutional Animal Care and Use Committee (IACUC) is composed of both active animal users and lay persons. Regardless of background, each member has a vote, so it is particularly important that the language of the application be understood by all. This applies to all sections of the application, but it is especially important that the goals and justifications of the proposed research be spelled out in the clearest possible terms. NOTE: Upon approval, this protocol will become a public record, so do not disclose proprietary information.

A. Provide a brief (300 words or less) synopsis in **NON-SCIENTIFIC TERMS** of proposed research.

The results of these experiments will provide a deeper understanding of how magnetic micro-stimulation of the cerebral cortex relate to subjective perception and will lay the foundation for implementing sensory prostheses. The prostheses will be developed in non-human primates as a precursor to applying a similar approach in humans. The rationale of the prosthesis is to magnetically micro-stimulate regions such as the primary visual cortex, an area of the cerebral cortex that processes visual input from the eyes. If this visual cortex can be properly stimulated in real-time, then patients who are blind from trauma, retinitis pigmentosa, macular degeneration, diabetic retinopathy or glaucoma could have limited vision restored.

We have also provided proof-of-concept for electrical micro-stimulation of the primary visual cortex serving as the basis for a vision prosthesis. The new work will determine the optimal way to micro-stimulate the visual cortex using magnetic stimulation. This work will allow us to improve the quality of vision restored to the profoundly blind.

B. PLANNED USE OF ANIMALS. Begin with a clear **statement of purpose** and briefly provide **background** information and **references** to previous work (especially if this is a renewal protocol). Include a clear description of the **experimental design** for all animal experiments planned and explain **why** the experiments must be performed. It is critical that for each procedure you provide a detailed sequence of events that effectively describes what happens to the animals from acquisition to euthanasia (if applicable). As the focus of the IACUC protocol is on animal use, do not simply cut and paste research objective statements from grant proposals. Flow charts, diagrams or tables are strongly recommended for complicated experimental designs. State how the research is expected to benefit the human community, the animal community, and/or society as a whole. **Details regarding surgical procedures, drug treatments, and field techniques are not necessary, as they will be addressed later in the form.**

For the development of sensory prostheses, we covertly substitute magnetic micro-stimulation of the cerebral cortex for the presentation of sensory stimuli and use the animal's behavior responses to indicate its perceptions. Using this technique, we can develop algorithms for encoding sensory input into patterns of cortical micro-stimulation that can evoke visual perceptions. We will use one animal in this study. The animal will receive pedestal implants for head fixation, as well as a chronically implanted access chamber over the primary visual cortex. The micro-coils will be acutely placed into the primary visual cortex during each experimental session. The micro-coils are very similar in design to the micro-electrodes commonly used to perform electrophysiological recordings. All guide tubes (also referred to as trocars or cannulas) and micro-coils will be handled, sterilized, and disposed of in accordance with the Trocar Use SOP. The animal used under this protocol will be housed and tested in a purpose-built primate research area within the vivarium. Whenever possible, each primate will be pair housed with at least one other conspecific. All animals are allowed time for contact, play, grooming, etc., except in the intervals immediately after implant surgeries. This housing situation will provide for the animal's psychological well-being. All phases of this protocol are undertaken in collaboration with the veterinary staff, who are consulted

on designing routine care, and who are available for any necessary medical invention.

The general overall timeline of events from acquisition to training is shown in the table below.

Estimated duration	Action
2 to 9 month	Behavioral training & timulation e periment

The timelines above reflect the estimated duration of events; actual duration may vary. For example, nonhuman primates (NHPs) may participate in multiple studies, which would increase their time training/recording by more than the months stated.

1. Training

For a naive animal, training begins by familiarizing them with the testing room and the personnel working with them. For additional information on the pole and chair training process, see the IACUC approved Standard Institutional Guidelines (SIGs) “NHP Pole and Collar Shaping plan” and “NHP Chairing Shaping Plan”. The animals are given treats while in the testing room as well as water/juice rewards during the experiment. While a monkey is seated in the chair, the experimenters will gently touch and groom them, a natural primate behavior, so that they become comfortable with human tactile interaction. Once the monkey is comfortable sitting in the chair for at least 40 minutes, they will be introduced to the behavioral apparatus. They soon discover that interactions with the behavioral control apparatus results in a squirt of fruit juice or water (0.2-1.0 ml.) from a spigot in front of the mouth, which is delivered automatically by an electronically operated solenoid valve. As soon as they appreciate the association between the behavioral control apparatus and the liquid reward, the criteria for reward are changed, such that more specific behaviors are required to get the reward. The shaping of the task performance is done slowly by degrees as the animal learns, so that the desired task performance is built up gradually over time with minimal frustration to the animal.

2. Tasks

The monkeys will perform several tasks that all consist of responding to visual stimuli on a display screen by looking at the stimuli and releasing buttons. The following are examples of these tasks described in detail.

Gaze Fixation Task: The monkeys will be trained to perform a fixation task while seated in a custom-built primate chair that allows for restraint of the head but free movement of both arms. The experimental setup will have two capacitance switches so that when the monkey's hands are placed upon them its arms will be at its sides with a natural and comfortable elbow angle. These switches have no moving parts and will be tuned so that the animal's hands must be held within 3mm of the active surface to trigger the switch. Placed 30cm distant from the monkey’s eyes, directly in front of the animal, will be a 20" video screen, which will be used to display visual targets. The direction of the monkey’s gaze will be monitored using a camera attached to the primate chair. When the monkey places both of its hands upon the capacitance switches a small (1 visual degree) visual will appear in the center of the video screen. The monkey will reflexively look at the target. When its direction of gaze is within 2 degrees of the center of the target it will be given a juice reward. The monkeys will quickly associate looking at the target with the juice reward. Initially the monkey will be rewarded the moment that its gaze enters the 2-degree window. Over time we will slowly increase the length of time that the monkey must fixate on the target in order to receive its reward. Monkeys will routinely fixate on a target for several seconds to obtain a reward. We will also train them to maintain fixation even when distracter stimuli are presented in the peripheral visual field, and during some tasks they will be trained to make eye movements to targets presented in the peripheral visual field.

Sensory Tasks: Once the monkey has mastered the ability to maintain fixation in the presence of distracters, we will train them in a forced-choice detection task. In this task they will be trained to respond to the presence of the distracters – changing the distracters to behaviorally relevant stimuli. The monkeys will be required to fixate on

the stimulus presented at the center of the screen for a variable period of time (500 – 2000ms). On some trials during this time period, a target stimulus will appear in the para-foveal space and in other trials no stimulus will be presented. The monkey will be trained to release the switch held by its right hand in order to indicate that it has perceived a stimulus and to release the switch held by its left hand to indicate that it did not perceive a stimulus. At the end of the trial a tone will indicate to the monkey that it should make its response. The monkey will also be trained to look where the visual stimulus appeared. The monkeys will be required to perform these tasks, but with the perceived sensation arising from magnetic micro-stimulation via the micro-coils of the visual cortex rather than through the normal sensory pathways. Stimulus pulse waveform was a half-period of 1-kHz sinusoid waveform. The amplitude of sinusoids from the function generator ranged from 0-3 V. The output of the amplifier for sinusoids was 0-8.61 V. The brief pulse of electricity in passed through the micro-coil which generates a small magnetic field in the visual cortex. There is no electrical connection to the neural tissue. We hypothesize that the small magnetic field will stimulate nearby neurons and result in the perception of a small point of light. Electromagnetic stimulation of the primary visual cortex has been performed in multiple human and nonhuman primate studies and results in the perception of small spots of light called phosphenes. The amplitude of the micro-stimulation is started very low and gradually increased until the animal reports that it sees a phosphene. Electromagnetic stimulation of the visual cortex does not generate noxious or distressing stimuli.

C. RATIONALE FOR INVOLVING ANIMALS AND THE APPROPRIATENESS OF THE **SPECIES AND NUMBER** USED. Keeping in mind the principles of the "3 R's" (Refinement, Reduction, and Replacement), answer the following:

1. Why must live vertebrates be used in this study?

We are developing neural prosthetics for implementations in human patients, so an accurate model of human neural systems is required. No lesser animal model, in vitro model, or computer simulation can replace the nonhuman primate pre-clinical testing.

For the purpose of developing sensory prostheses, computer modeling, cell cultures, or other non-organismal preparations are inadequate as they cannot indicate subjective visual perception.

Invertebrates cannot be used for these studies as they cannot be trained to perform the complex behavioral tasks (involving combinations of eye and limb movements) that are required. The possible alternatives to single-cell recordings would consist of computer simulations and recording from neurons in neural tissue culture, brain slice preparations, and human beings. Computer simulations are inadequate because they cannot provide any new information about neural responses that were not already known and programmed into the simulation. While we may also employ computer simulation and modeling techniques in this study, such methods cannot substitute for actual observations. Tissue cultures and brain-slice preparations of cortex are not a possible alternative because connections to the eyes and other cortical areas would be lost. Experiments on human beings would involve surgery, would need to be carried out under anesthesia, would carry considerable risks to the human subjects, and would have few direct benefits to the patient.

2. Why are you using the requested species rather than other species?
   The goal of this project is to understand the encoding of sensation in the central nervous system. This understanding will be used in the development of a vision prosthesis for the treatment of blindness. We use *Macaca mulatta* monkeys as laboratory animal models for humans because their brain anatomy and behavior are sufficiently close to those of humans and they are the most widely and historically used macaque for this type of research. A primary reason for using macaques is that they are capable of being trained to perform the tasks required in this proposal. Additionally, the macaque visual system is sufficiently similar to that of humans to make these experiments relevant to implementing prostheses for human patients. At the level of systems neurophysiology and human-like behavior at which this work will be done, no other animal except a phylogenetically higher primate is suitable.

3. What is the rationale supporting the numbers of animals proposed? Typically, a power analysis should be performed to support the proposed sample sizes. A table depicting the number of animals to be used is required.
   A single *Macaca mulatta* monkey will be used during the proposed experiments. Since we will be performing electrophysiological recordings from and stimulation of the cerebral cortex and peripheral nerves, the statistics needed to validate results will be determined by the number of behavioral trials executed, rather than the number of animals used. The data will be analyzed using widely accepted and commonly used statistical tests (e.g., ANOVA, regression analysis). Statistical significance can be achieved with hundreds of behavioral trials.

4. What refinements, if any, have been made to reduce the number of animals used and the potential detrimental effects on the study animals?
   We are using state of the art technology to minimize the invasiveness of procedures, including using a minimally invasive head-restraint system instead of the commonly used larger acrylic head-cap. We use a smaller, custom-fitted cortical chamber that is biocompatible and will not require the need for dental acrylic to stabilize. We also use optical imaging instead of scleral eye-coils for monitoring eye position [redacted]

   In addition, we have continuously updated our surgical and analgesia regimens to reduce the time in surgery and close any windows in which there is likelihood for pain without adequate analgesia. We also train the NHPs to minimize stress during chair restraint (see IACUC-approved SIGs "Pole and Collar Shaping Plan" and "Chairing Shaping Plan"), we maintain implants to minimize infection (see IACUC SIG "NHP Implant Maintenance"), and have a well-designed psychological and environmental enrichment program to minimize stress to the animals.

III. EMERGENCY CONTACT

A. Who should be contacted in case of an animal emergency? **Note: This information will be redacted if this protocol is requested as a public document.**

Name:
Office Phone #
Home Phone #
Cell Phone #: [redacted]

IV. DUPLICATION AND ALTERNATIVES PLEASE READ ALL INSTRUCTIONS.

The Animal Welfare Act requires that you document your justifications with data from **two** or more sources. **One source** must **be a set of searches of a relevant database: name the database searched, the keyword and keyword combinations searched, the date the search was performed and the date range searched. The second source can be a set of searches of a second relevant database, or consultation with a laboratory animal science veterinarian, or courses/meetings/consultations with qualified personnel.** Sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge of the availability of alternatives in the specific field of study. Examples of appropriate databases to search include PUBMED, Web of Science, or Animal Welfare Information Center (AWIC – recommended for USDA-covered species https://www.nal.usda.gov/awic/databases).

A. Provide the following details for the most recent literature search used to explore for duplicative research. (The literature search documents that the research will not unnecessarily duplicate previous research). **Teaching protocols do not need to conduct this search.**

Date that search was conducted (*Must be within 60 days of the IACUC review date*): 20-Jan-2021
Database(s) used: Pubmed
Publication years covered by the search: 1959 - 2020
Keyword combinations used: vision prosthesis, magnetic stimulation, cerebral cortex. These key words were searched in various combinations of 1 – 3 words.

B. Provide the following details for the most recent literature search used to explore for **alternatives to animal use** and **alternatives to painful procedures.** Alternatives should be considered for any aspect of the protocol that may cause more than momentary or slight pain or distress to the animal. Alternatives to be considered include those that would: 1) refine the procedure to minimize discomfort that the animal(s) may experience; 2) reduce the number of animals used overall; or 3) replace animals with non-animal alternatives (e.g., computer models or tissue culture). **All protocols (research and teaching) MUST conduct this search.**

Date that search was conducted (*Must be within 60 days of the IACUC review date*): 20-Jan-2021
Database(s) used: Pubmed
Publication years covered by the search: 1981-2020
Keyword combinations used: Pain; distress; alternative; anesthesia; analgesia; non-animal model; restraint; macaque; rhesus; monkey; head fixation; water restriction; reduction, refinement, and replacement These key words were searched in various combinations of 2 – 5 words.

C. **Results of literature search for alternatives**: Comment on the application(s) of any identified alternatives (found with your search terms, including how these alternatives may be or may not be incorporated to modify a procedure to either lessen or eliminate potential pain and distress. **All protocols must complete this section and must describe how the literature search results relate to painful procedures and alternatives to animal use.** You must include sufficient information for the IACUC to determine that a reasonable, good faith effort was made to determine the availability of alternatives. If the search identified any alternative methods (ones that could be used to accomplish the goals of the animal use proposal), you must clearly explain and justify why this alternative cannot be used.

For instance, if your search terms retrieved eight publications, summarize how many of those described alternatives to painful procedures and the use of animals.

The searches listed above revealed no viable alternatives for the procedures used in the current research proposal but reinforced our ideas of maximizing the animals' wellbeing through proper handing and environmental enrichment, as well as improving surgical technique and implant design. Databases were searched for any methods which would replace, refine, or reduce the use of animals in these studies. The search spanned multiple years and search terms were presented in multiple appropriate combinations. The search terms included key

words taken from the title of, and throughout, the protocol; the animal species used; the system being studied, and the three Rs (reduce, refine, replace – as defined in international legislation and regulation of the use of animals in scientific research).

D. Describe any other procedures (e.g., participation in meetings, review of journals) that are used to explore and evaluate alternatives: The PI, lab manager, post-docs, and graduate students regularly attend national meetings and discuss with colleagues recent updates in technology and methodology for these experiments. Additionally, they remain up to date with the scientific literature on new and alternative procedures.

E. Does this research replicate previous work? **(Your answer will be based in part on the literature search above.)**

☒ No. Proceed to section **VI.**

☐ Yes. Explain why the replication is necessary:

☐ Not applicable. This is a teaching protocol.

V. CATEGORY OF PAIN OR DISTRESS

For non-USDA covered species, answer question A only. For USDA covered species, answer question B only. USDA covered species are all mammals EXCEPT laboratory mice and rats bred for research. All other rodents, including wild mice and rats, are covered.

A. Do the procedures in this protocol have the potential to involve more than slight or momentary pain or distress that will **NOT** be relieved with anesthetics, analgesics, tranquilizer drugs, or other method for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

B. Using the table below, list all USDA covered species to be used in the proposed study and indicate the number of animals to be used under each USDA pain category. For an animal undergoing multiple procedures, include the animal under the highest level of pain/distress expected for that animal.

USDA Covered Species	Number per USDA Category*				Total number of animals requested
	B	C	D	E	
Macaca mulatta			1		

*USDA PAIN CATEGORIES: (see http://researchintegrity.asu.edu/animals/forms for a more complete description of the below categories)

Classification B: Includes animals that are used solely for breeding or are being acclimatized or held for use in teaching, testing, experiments, research, or surgery but have not yet been used for such purposes.

Classification C: Includes the use of animals in procedures involving no, momentary, or slight pain or distress (e.g., non-invasive parenteral drug delivery, peripheral blood collection, euthanasia, short-term manual or chemical restraint, toe clipping).

Classification D: Includes the use of animals used in procedures that could cause pain or distress but appropriate anesthetics, analgesics, and/or tranquilizing drugs or other methods for relieving pain or distress are used (e.g., surgery, perfusion, administration of irritating chemicals, humane endpoint euthanasia).

Classification E: Includes the use of animals in procedures that have the potential to involve pain or distress that will **not** be relieved with anesthetics, analgesics, tranquilizer drugs, or other method for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia).

VI. ASSURANCE:

The information contained herein is accurate to the best of my knowledge. I have carefully compared the proposed work with the current state of knowledge in this field by reviewing the literature and it is my professional opinion that the proposed work meets high standards of scientific merit. If the study involves pain and distress to the animal, whether or not it is relieved by anesthetics or analgesics, I have (1) reviewed the literature related to this work and have found no significant studies which could make this protocol unnecessarily duplicative, and (2) considered alternatives to animal use and found none available, as described above. Procedures involving animals will be carried out humanely and all procedures will be performed by or under the direction of trained or experienced persons. Any revisions to animal care and use in this project will be promptly forwarded to the Institutional Animal Care and Use Committee for review. Revised protocols will not be used until Committee clearance is received. The use of alternatives to animal models has been considered and found to be unacceptable at this time.

The principal investigator, by signing below, and the IACUC recognize that other medications may be given to the animals for veterinary care purposes. This includes the humane euthanasia of animals in uncontrollable pain or distress as determined by the Attending Veterinarian or the Clinical Veterinarian acting for the Attending Veterinarian. However, the veterinarians will make all efforts to contact and discuss the case with the Principal Investigator or designee prior to making a unilateral decision.

[redacted] 20-Jan-2021

Principal Investigator –Print | Date

Principal Investigator Signature | Date

NOTE: Principal investigators must submit a current curriculum vitae or biosketch that reflects their most recent pertinent experience.

PERSONNEL CHART

ASU requires that all personnel engaged in animal research or teaching be qualified through training or experience in order to conduct the work humanely. The IACUC requires the following training:

- **Level I Basic** – Required of ALL participants (must be renewed every 4 years)
- **Level II Species-Specific** – Required for each participant that will have direct contact with that species (must be renewed every 4 years)
- **Level III Hands-on Training** – Required to perform specific procedures independently; Level III Certification form must be submitted to the IACUC office by the person providing the training within 5 days of the training

You can access the training modules at https://asu.co1.qualtrics.com/jfe/form/SV_b2b2XRXRRs1309f. See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

All procedures MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel that are not Level III certified are supervised at all times.

Name	Title	ASURITE name	Role in Protocol: What procedures will each person be doing on live animals under supervision only?	Role in Protocol: For which procedures is each person Level 3 certified at the time of protocol submission?	Species with which individual will have direct contact ("none, "all", or list species)	FOR IACUC USE ONLY: Training Confirmation
[redacted]	PI	[redacted]		Responsible for overall conduct of all studies, including performing surgeries, poling, handling, restraint, implant maintenance, and training.	Macaca mulatta	6/2017 OHSP
	Lab Coordinator		Assistance with surgical procedures	Lab management, running behavioral tasks, poling, handling, restraint, implant maintenance, and training.	Macaca mulatta	11/2018 OHSP L3
	Research Associate			Primary data collection, poling, handling, restraint, training, perform surgeries, implant maintenance	Macaca mulatta	11/2018 OHSP L3

For each individual, describe the individual's years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

[redacted] Has been working with and performing surgical procedures on nonhuman primates for approximately 18 years. He has received animal care and surgical training from multiple veterinarians, physicians, and surgeons over this time.

[redacted] has more than 12 years of NHP handling and training experience, including 6 years with rhesus macaques.

[redacted] has approximately 10 years' experience handling, training, and assisting/performing surgeries with rhesus macaques.

DETAILED USE OF ANIMALS

This section must be completed for each species used.

(additional Detailed Use of Animals forms can be found at https://researchintegrity.asu.edu/animals/forms)

Common Name: Rhesus macaque

Scientific Name: *Macaca mulatta*

I. ANIMAL INFORMATION

A. Is this a threatened or endangered species?

☒ No. Proceed to section I. B.

☐ Yes. Describe why this work must be done on this species and why the project will not have a significant negative impact on the species:

B. Maximum # of animals to be used over the 3-year life of the protocol: 1

C. Sex: Male Age or Weight Range: up to 20 kg

D. Source (e.g., commercial, in-house breeding, captured from wild): Commercial facilities

E. List all labs and/or rooms **outside of the ASU centralized vivaria** where you intend to keep or use live animals in connection with the animal use covered under this protocol. This list is for IACUC information to assure each location is inspected semi-annually. **Listing rooms here does not assure approval of this space for use.**

Building	Room #	Max Length of Stay	Method of Transport	Purpose
[redacted]		2 Hours	DACT Truck	MRI
		1 Hour	DACT Truck	CT Scan

F. If you use DEA-controlled substances, list the location where they are stored (building and room number). If you acquire controlled substances from DACT for same day use, state this. The IACUC is required to inspect all controlled substance storage locations semi-annually. DEA controlled substances are either administered by DACT veterinary staff or provided on a treatment by treatment basis. Therefore, the lab does not maintain any controlled substances.

II. MAJOR CATEGORIES OF USE

A. Will animals be immunized for production and harvesting of antibodies?

☒ No. Proceed to section II. B.

☐ Yes. Complete the following table.

Injection:

Volume of injectate	Adjuvant	Route	Min Frequency	Ma # of injection

Collection: If terminal, check here ☐ otherwise complete the following.

Route	Max. Volume	Min. Frequency	Max. # of collections

B. Will tissues, blood, or other body fluids be harvested (other than for antibody production)?

☐ No. Proceed to section II. C.

☒ Yes. Will tissues, blood, or other body fluids be collected post-mortem only?

☒ Yes. Proceed to section II.C.

☐ No. Complete Appendix 1: Antemortem Specimen Collection.

C. Will animals be food restricted (calorically or specific constituents) other than for surgical procedures?
☒ No. Proceed to section II. D.
☐ Yes. [note: restriction paradigms exceeding a single 24-hr period must follow the ASU IACUC Standard Institutional Guideline for Food and Water Restriction available at https://researchintegrity.asu.edu/index.php/animals/procedures-library-and-guidelines

1. What are the restriction parameters? Provide scientific justification and include the length of restriction.

2. How will you monitor for negative effects of food restriction (include information on how you will account for animal growth)?

D. Will animals be water restricted?
☐ No. Proceed to section II. E.
☒ Yes. [note: restriction paradigms exceeding a single 24-hr period must follow the ASU IACUC Standard Institutional Guideline for Food and Water Restriction available at https://researchintegrity.asu.edu/index.php/animals/procedures-library-and-guidelines

1. What are the restriction parameters? Provide scientific justification and include the length of restriction.
Water will be available only at limited times during the day: first during the behavioral sessions, and second at the end of the day. On days when animals are not working, their water allotment is split with one half administered in the AM and the second in the PM. Amounts of water provided will vary with the animal's weight, current work regime, and habits. This water restriction paradigm is used to provide an incentive for work. Details are found in the IACUC SIG "NHP Fluid Regulation".

Monitoring for negative health effects will remain the same as outlined in the NHP Fluid Regulation SIG. A drop of more than 10% body weight from the animal's baseline weight will be reported to the veterinarian and the animal will be provided with extra water. Moistened biscuits, and/or produce/forage with a higher fat content may also be provided. The course of action will be decided in consultation with the veterinarian.

2. How will you monitor for negative effects of water restriction (include information on how you will account for animal growth)?
Details regarding monitoring of health and allowances for growth are provided in the available IACUC-approved SIG, "NHP Fluid Regulation".

E. Will animals be exposed to trauma, injury, burning, freezing, electric shock, UV radiation, magnetic fields, lasers, loud noise, or other physical agents that might cause distress?
☐ No. Proceed to section II. F.
☒ Yes. List and justify each exposure.
Provide scientific justification: MRI scans involve strong magnetic fields, and precautions are made to ensure that no incompatible metals are present in the room during the scan. Noise levels inside an MRI machine typically vary from 65 to 95 dB, and intermittent spikes of ~110 dB may be produced. MRI scans will be performed under sedation or anesthesia, and ear protection using ear plugs or gauze/cotton will be placed in the animal's ears to prevent damage and mitigate distress.

F. Will animals be exposed to environmental stress (e.g., non-natural temperature exposure, prolonged physical restraint, forced exercise)?
☐ No. Proceed to section II. G.
☒ Yes. List and scientifically justify each exposure.
Partial restraint of each animal during experimental sessions is necessary to ensure the accurate recording of eye movements and to prevent any injury to the animal while they are connected to the data acquisition equipment. During experiments animals will be seated in a primate chair and their heads will be fixed in place (described below).

Primate chair restraint:
The animal wears a nylon or aluminum collar that attaches to the chair by a collar latch. The latch secures the monkey in the chair. The NHP is trained according to the IACUC SIGs "Pole and Collar Shaping Plan" and "Chairing Shaping Plan". The chairs are designed with many adjustable parts, and

each chair is fitted to the monkey's individual size. Care is taken to ensure that the animal is seated comfortably, and no points of pressure exist between the animal's body and the chair. The animal is free to move its limbs and torso during the period of head restraint and the animal's head is not restrained during transport.

The monkey also wears an aluminum halo that is affixed to the head by posts described in the surgical procedures section. The halo is then connected to an attachment that connects to the chair or the experimental setup table so the head cannot move. The head restraint is necessary in our tasks as the monkey's head must be perfectly still for recordings and to monitor eye position. The animal is always monitored during partial restraint to ensure that it is not uncomfortable or stressed, as indicated by struggling or frequent changes of position in the chair. Monitoring during the experimental session is performed using an infrared video camera; this allows us to monitor the animal even in the dark. In our extensive experience we find that the animals tolerate the partial restraint very well, and will even sleep when they are not asked to work. If an animal becomes tired or uncomfortable, they will typically not perform the behavioral task required. Thus, the length of the recording session may be dictated by the animal.

Animals are restrained in primate chairs typically 2-8 hours per day, five to seven days per week. Each animal has one or two investigators responsible for restraining (chairing) the animal during experiments. Training sessions during which the animal is required to participate in behavioral tasks last between 2 to 8 hours. Experimental sessions are generally 4 hours, and on rare occasions, if the animal continues to work, can be extended to 8 hours. Some experiments do require continuous data collection for up to 6 hours without interruptions. However, it is more typical that the animal has several periods of rest while restrained. Typically, animals sleep during these periods. Often, if the recording session is more than 6 hours, the animal is given treats during a break of up to 30 minutes after the first 6 hours. Restraint is limited to the duration of the daily recording experiment and every effort is made to reduce time spent in the chair. Animals are not chaired between experimental sessions, except to monitor their weight as well as facilitate implant cleaning and any medical treatments that may be required by the veterinarians. The animal's head is restrained only during experimental sessions or during implant maintenance cleaning.

G. Will animals undergo surgery?
- ☐ No. Proceed to section II. H.
- ☒ Yes. Complete Appendix 2: Surgical Procedures.

H. Will any animals have a device (e.g., thermocouple, cannula, electrode) that extends chronically through the skin?
- ☐ No. Proceed to section II. I.
- ☒ Yes. Describe wound management measures to minimize chances of infection around the device where it penetrates the skin:

Connectors and chambers will extend through the skin. The craniotomy chamber will be sealed at the end of each experimental session with either a cap that is screwed onto the top of the chamber or a silicone elastomer plug. Elastomer plugs have been shown to prevent the buildup of granulation tissue in recording chambers, which we would like to avoid. The article referenced below discusses how this method has worked successfully in three non-human primates for up to 21 months and was also left in the chamber untouched for several months. When the plug is first applied, we will check it every day to monitor for fluid buildup. If fluid is seen, we will remove the plug, clean the chamber, and replace with a new plug. If no fluid is seen for 1 week, we will check for fluid at least once every 7 days. For the monkeys that are recording, we will remove the plug before recordings, record, clean the chamber, and then apply a new plug.

Reference for silicone elastomer- [redacted]

All open wounds will be managed with routine cleaning (minimally once every seven days) using a disinfectant agent such as chlorhexidine, and all appliances will be routinely inspected for signs of infection. In the case of infection, treatment will involve one or more treatments such as debriding, flushing, treatment with topical antibiotics, or treatment with systemic antibiotics, in consultation with the DACT veterinarians.

See the IACUC SIG, "NHP Implant Maintenance" which describes our laboratory SOP for dealing with devices which extend through the skin.

I. Will animals need any special husbandry considerations, including but not limited to single housing individuals of social species (e.g., rodents), altering standard cage type, cage change frequencies, housing temperature, or lack of enrichment?

☐ No. Proceed to section II. J.
☒ Yes. Describe special procedures and provide scientific justification:
Animals are pair housed except for recovery periods following surgery or if there is a danger of the paired animals injuring one another. Since we use such small number of animals, a suitable pairing partner may not be available.

J. Will any work be conducted in the field (this includes field experiments or the capture of animals to be used in laboratory experiments)?

☒ No. Proceed to section II. K.
☐ Yes. Complete Appendix 3: Field Research.

K. Will any animals need to be individually identified?

☐ No. Proceed to section III.
☒ Yes. Describe the marking technique to be used, why that technique was chosen, how it will be performed, and on what age range of animals?
USDA tattoo number located on chest or inguinal area. Animals either have the tattoo upon arrival or are tattooed by DACT staff during quarantine. Touch ups may be done while sedated/anesthetized (e.g., for TB testing), and the hair in the region is shaved as needed to maintain visibility of the tattoo.

III. CHEMICALS AND OTHER POTENTIAL HAZARDS

(If you answer yes to any of the following questions, this information may be forwarded to another oversight unit to aid you in assuring safe practices. Approval by these units or additional training may be required prior to using any of these materials)

A. Will drugs or chemicals be used with animals?

☐ No. Proceed to section III. B.
☒ Yes. For each drug or chemical, list the agent, dose, route, purpose, and grade in the table below:

Agent	Dose	Route	Purpose	Frequency	Pharmaceutical grade (Y/N)?	Is this a DEA controlled substance (Y/N)?
Acepromazine	0.1-0.5 mg/kg	IM	Sedation for head fixation	As needed	Y	N
Atropine	0.02-0.05 mg/kg	IM	Reduce respiratory secretions and prevent bradycardia	Once, as needed	Y	N
Betadine	N/A	Topical	Clean implants; Disinfect surgical sites	As needed	Y	N
Bupivacaine	1-2 mg/kg	SC	Local anesthetic	Once, as needed	Y	N
Buprenorphine	0.01-0.03 mg/kg	IM or SC	Analgesic	Every 6-12 hours, based on vet assessment	Y	Y
Buprenorphine SR	0.2 mg/kg	SC	Analgesic	Once, based on vet assessment	Y	Y

Cefazolin	20-25 mg/kg	IV or IM	Antibiotic	Every 4 hours intra-operatively, BID based on vet assessment	Y	N
Cephalexin	20-30 mg/kg	PO	Antibiotic	BID based on vet assessment	Y	N
Chlorhexidine	N/A	Topical	Clean implants; Disinfect surgical sites	As needed	Y	N
Dexamethasone	0.25-2 mg/kg	IM or IV	Reduce inflammation	As needed based on vet assessment	Y	N
Dexmedetomidine	0.02-0.05 mg/kg	IM	Sedative	Once, as needed	Y	N
Doxapram	2 mg/kg	Topical (tongue) or IV	Stimulate breathing	As needed based on vet assessment	Y	N
Enrofloxacin	5 mg/kg	PO or IM	Antibiotic	SID or BID, based on vet assessment	Y	N
Epinephrine	0.2-0.4 mg/kg	SC, IM or IV	Stimulate heart, vasoconstriction	As needed based on vet assessment	Y	N
10% Formalin ± 20% glycerine	4L	IV	Perfusion	Once	N	N
Gelfoam	Cut to size	Topical	Hemostasis/Seal surgical holes	Once, as needed	Y	N
Glycopyrrolate	0.005-0.01 mg/kg	IM	Reduce respiratory secretions and prevent bradycardia	Once, as needed	Y	N
Hydrogen peroxide	N/A	Topical	Clean implant	As needed	Y	N
Hydromorphone	0.05-0.2 mg/kg	SC, IM or IV	Analgesic	As needed, based on vet assessment	Y	Y
Isoflurane	1-5%	Inhalation	Anesthetic	Continuous, during surgery	Y	N
Ketamine	3-15 mg/kg	IM	Sedative	Once, as needed	Y	Y
Lactated Ringer's Solution	5-15 ml/kg/hr	IV	Fluid support	Continuous, during surgery, as needed	Y	N
2-5% Lidocaine	1-4 ml	Nerve injection via catheter	Nerve block	Once a day, during data collection	Y	N
Lidocaine containing gel/cream	Dab	Topical	Local anesthetic	As needed	Y	N

Mannitol	0.25-2.2 g/kg over 20 minutes	IV	Reduce intracranial edema	As needed	Y	N
Meloxicam	0.1-0.2 mg/kg	PO or SC	Analgesic, reduce inflammation	Once a day, based on vet assessment	Y	N
Metoclopramide	0.2-0.5 mg/kg	IM	Antiemetic	As needed, based on vet assessment	Y	N
Midazolam	0.05-0.5 mg/kg	IM or IV	Sedative, anticonvulsant	As needed	Y	Y
Ophthalmic ointment	Dab	Topical	Prevent corneal desiccation	Once, as needed	Y	N
Oxymorphone	0.7-0.15 mg/kg	SC, IM or IV	Analgesic	As needed, based on vet assessment	Y	Y
Pentobarbital-containing euthanasia solution	86-120 mg/kg	IV	Euthanasia	Once	Y	Y
Phosphate buffered saline	4L	IV	Perfusion	Once	N	N
Propofol	2-5 mg/kg Bolus 0.2–0.6 mg/kg/min CRI	IV	Sedative	Once, as needed Continuous, as needed	Y	N
Sevoflurane	1-8%	Inhalation	Anesthetic	Continuous, during surgery	Y	N
0.9% NaCl Solution	5-15 ml/kg/hr	IV	Fluid support	Continuous, during surgery, as needed	Y	N
3% NaCl Solution	250 ml bolus over 30 minutes	IV	Reduce intracranial edema	As needed	Y	N
Tiletamine/Zolazepam	1.5-10 mg/kg	IM	Anesthetic	As needed	Y	Y
Tramadol	1-2 mg/kg	PO	Analgesic	Once to twice a day, as needed based on vet assessment	Y	Y
Triple antibiotic ointment/Silver sulfadiazine	Dab	Topical	Antibiotic	As needed	Y	N

1. For each drug or chemical that is not pharmaceutical grade, indicate whether no pharmaceutical grade equivalent exists or provide scientific justification for using the non-pharmaceutical grade product.

Phosphate buffered saline, 10% formalin, and 10% formalin with 20% glycerin are not available in a pharmaceutical grade. These will only be used in conjunction with perfusion as a terminal procedure.

B. Does this project involve transgenic animals?
☒ No. Proceed to section III. C.
☐ Yes. List the strains, any special care needs, and any expected clinical signs that are associated with the strain. Transgenic animals need to be covered by an IBC disclosure.

C. Does this project involve the use of biohazardous agents in animals (microorganisms, microbial toxins, recombinant DNA)?
☒ No. Proceed to section III. D.
☐ Yes. List the agent, as well as concentration, dose, and route if applicable.

Agent	Concentration	Dose	Route	ADMIN. USE ONLY	
				ABSL	IBC # if Req'd

D. Does this project involve irradiation or the use of radiological material in animals?
☐ No. Proceed to section III. E.
☒ Yes. List the agent, dose, route, and purpose in the table below:

Agent	Dose	Route	Purpose
X-rays (CT scan and radiographs)	CT scan: ~2 mGy Radiographs: Various (average ~0.01-0.2 mGy per radiograph)	CT scan – Head Radiographs - Various	Diagnostic imaging/Surgical planning

1. Provide the date of **Radiation Safety Committee** approval:

E. Describe any health hazards to **researchers** and include a description on how the risk is mitigated or managed:
Risk of bites, scratches, or Herpes B (Herpes B virus is not being used in animals but can be transmitted to personnel if there is an NHP bite/exposure). Risks are mitigated with the use of additional PPE (tyvek sleeves, eye protection, double gloves), NHP primate certification, annual B Virus training (including Bite/Scratch policy), proof of 2 MMR vaccines or a measles titer, annual TB screening, dosimeters and lead shielding during radiographic procedures, and ear protection during MRI scans.

F. Describe any health hazards to **animals** and include a description on how the risk is mitigated or managed:
Zoonosis such as TB, measles, flu are concerns to spread from human to monkey. Before working with an NHP, all researchers are required to show proof of 2 MMR vaccines or a measles titer and annual TB screening. All people interacting with the monkeys are also required to wear a surgical mask to prevent the spread of those infections.

IV. DETRIMENTAL SEQUELAE

A. Will animals possibly experience clinical signs intentionally or as a possible side effect of the study?
☐ No. Proceed to section V.
☒ Yes. Complete the following.

Possible Clinical Effect	Probability of Occurrence	Treatment
Neurologic symptoms such as: paresis, spasticity, paralysis	1%	*Ad libitum* access to fluids, rest, medications/treatments, or removal from study per veterinary recommendation
Infection	5%	*Ad libitum* access to fluids (systemic), antibiotics

		(systemic or local), other medications/treatments per veterinary recommendation
Dehydration from fluid regulation	1%	*Ad libitum* access to fluids
Implant Infection	10%	Clean with hydrogen peroxide, betadine or chlorhexidine. Other treatments per veterinary recommendation. See IACUC-approved SIG "NHP Implant Maintenance".
10% body weight loss from fluid regulation	10%	Refer to the SIG "NHP Fluid Regulation" for details on modifications for weight gain.
Loss of appetite due to fluid regulation	50%	Avoid dehydration, high calorie supplements, monitor body weight, moisten biscuits if needed to stimulate appetite, increase water allotment if needed
Post-operative pain	75%	Analgesia regimen, see IACUC approved SIG "Macaque Anesthesia/Analgesia/Antibiotic Regimens"

V. END POINT CRITERIA

A. What clinical signs will be used as a basis for removal of an animal from the study?
A body weight loss of 25% or greater (if animal began at an ideal or baseline body weight) that is nonresponsive to high calorie supplementation or other indicated treatment. This number provides us a substantial buffer (from 10% body weight loss to 25%) to correct whatever health issues an animal may be facing. It is our experience that an animal which has lost 25% or more of its body weight is on a terminal progression. Refer to the SIG "NHP Fluid Regulation" for how baseline body weight is obtained.

Any clinical disease that significantly impacts animal well-being and is unresponsive to aggressive medical treatment based on veterinarian input.

Major complications in a surgical procedure when non-responsive to aggressive medical and surgical intervention based on veterinary input.

VI. EUTHANASIA

A. List the primary method of euthanasia:
These animals may be euthanized for clinical reasons determined in consultation with the DACT veterinary staff (e.g., see end point criteria above), or in some cases because their tissue is needed for histological examination of implantation sites. In general our aim is to retire these animals to a primate sanctuary at the end of study.

If performed, euthanasia will primarily consist of injection of euthanasia solution (Pentobarbital solution) or exsanguination and perfusion with 10% formalin while under anesthesia in accordance with the IACUC SIG for perfusion.

For exsanguination and perfusion: The animal is first sedated with an appropriate sedative and anticholinergic (e.g., ketamine/atropine). They may also be administered an analgesic to prevent any pain felt by the sternotomy. The NHP is then deeply anesthetized. Once a deep plane of anesthesia is obtained, the animal is then exsanguinated via cardiocentesis, while 4L of PBS (phosphate buffered saline), followed by 4L of 10% formalin solution, and then by 4L of 10% formalin solution with 20% glycerin is pumped through the heart in order to fix the brain. In the event a perfusion is not necessary, a pentobarbital-containing euthanasia solution may be administered following sedation.

B. If using a chemical or gas, complete the chart below:

Various combinations of the following drugs may be used in coordination with euthanasia via injection of a euthanasia solution or perfusion.

Agent	Dose	Route	Is this a DEA controlled substance (Y/N)?	Secondary method used to confirm euthanasia
Pentobarbital-containing euthanasia solution	86-120 mg/kg	IV	Y	Thoracotomy or vital tissue harvest
Ketamine	10-15 mg/kg	IM	Y	Thoracotomy, perfusion, or vital tissue harvest
Midazolam	0.05-0.5 mg/kg	IM	Y	Thoracotomy, perfusion, or vital tissue harvest
Atropine	0.02-0.05 mg/kg	IM	N	Thoracotomy, perfusion, or vital tissue harvest
Glycopyrrolate	0.005-0.01 mg/kg	IM	N	Thoracotomy, perfusion, or vital tissue harvest
Isoflurane	3-5%	Inhalation	N	Thoracotomy, perfusion, or vital tissue harvest
Sevoflurane	5-8%	Inhalation	N	Thoracotomy, perfusion, or vital tissue harvest
Oxymorphone	0.07-0.15 mg/kg	IM	Y	Used in coordination with perfusion
Hydromorphone	0.05-0.2 mg/kg	IM	Y	Used in coordination with perfusion
Phophate buffered saline	4L	IV	N	Used in coordination with perfusion
10% Formalin ± 20% glycerin	4L	IV	N	Used in coordination with perfusion

C. If euthanasia is being done by a physical means (e.g., decapitation, cervical dislocation) without anesthesia, provide scientific justification:

APPENDIX 2: SURGICAL PROCEDURES

I. GENERAL INFORMATION

A. Species
Macaca mulatta

B. Surgical Procedure(s)
1) Implantation of pedestals for head fixation
2) Placement of posts for head fixation
3) Implantation of craniotomy chamber
4) Implant repairs
5) Implant removal
6) Vasectomy or Castration

Each animal will undergo several surgeries. The pedestal/post surgery will be performed first, followed by the craniotomy chamber surgery. Additional repair surgeries may be required to correct problems (e.g., replacement or relocation of a loose pedestal or array connector). These repair surgeries will be discussed and performed in consultation with the DACT veterinarians. Any new surgeries that are not already described in this protocol or any modifications to the surgical procedures as currently described will require an amendment approved by the IACUC.

C. Room/location of surgery
Surgical Suite, [redacted]

II. PRE-SURGICAL CARE

A. Will the animals undergo pre-surgical fasting?
☐ No. Proceed to section III.
☒ Yes. Provide the details:
The day before a scheduled surgical procedure, the NHP is offered its full diet allotment in the early afternoon, and any remaining diet is removed at the end of the day. The animal is then fasted overnight until the scheduled surgery the following morning in order to mitigate the risk of emesis and aspiration during the procedure.

III. SURGICAL PROCEDURE:

☒ Survival ☐ Nonsurvival

***Note:** A surgical checklist is required to be submitted for each survival surgery. A surgical checklist may be requested for nonsurvival surgeries.

A. Describe each surgical procedure (e.g., approach, tissue manipulation, closure):
In order to aid in the design and decide on proper placement of the craniotomy chamber, we may obtain MRI and CT images of each monkey. Please refer to the IACUC approved SIG "NHP Imaging" for details. If we are unable to obtain an MRI, we will use a stereotaxic atlas of the rhesus monkey brain to locate the coordinates needed for surgery.

Preoperative Care and Induction/ Post-operative closing:
The day before surgery the animal is fasted overnight to prevent emesis and aspiration. In general surgeries and procedures begin as early as possible to allow sufficient time for completion of the procedure and post-operative monitoring of the patient during hours that the veterinarian is on campus. The animal is sedated and anesthetized per the SIG "Macaque Anesthesia/Analgesia/Antibiotic Regimens". The animal's vital signs are monitored, a weight is obtained, and all information is recorded in the surgical anesthesia record. Ophthalmic ointment is placed in both eyes to prevent corneal drying. An IV catheter is placed to provide intravenous access in case of emergency and to deliver fluid therapy during the surgical procedure. Fluids are administered throughout surgery. The animal is intubated and placed under general anesthesia. Vital parameters such as ETCO2, ECG, body temperature, heart and ventilatory

rate, pulse oximetry, and blood pressure (direct or indirect) are monitored continuously. Some surgical procedures require that the animal's head be positioned in a stereotaxic frame to ensure that correct location of the brain structure to be studied is obtained. Lidocaine gel/cream is applied to the ear bars prior to use to provide local pain relief. After the head is shaved and scrubbed with novalsan/alcohol, a sterile field is established with the use of surgical drapes. For all procedures, the subcutaneous tissue and skin (if applicable) will be closed with an absorbable suture such as [redacted] or [redacted] (2-0 or 3-0) in addition to surgical skin glue unless otherwise directed by the veterinarian based on the circumstances.

Pedestal Implants (Head fixation)
This procedure provides mounting points for three pins that are installed to fix the animal's head. The pedestals are small (1.5 cm) multi-flange pods that are affixed flush with the skull using bone cortex screws. For each pedestal, an ~2 cm incision is made over the selected site while the animal is positioned in a stereotaxic frame, and the skin and muscle layers are progressively dissected to the skull. The area that will support the pedestal is then scraped with a periosteal elevator, and the pedestal feet are shaped to the profile of the skull. Once shaped, the skull will be lightly abraded around the profile of the implant to encourage osteogenesis, holes will be drilled for each of the legs of the pedestal, and it will be secured in position with bone screws. Finally, the incision will be closed with suture, staples, or skin glue based on veterinary recommendation. Once the animal has awakened, normal post-surgical protocol will be followed per the SIG "Macaque Anesthesia/Analgesia/Antibiotic Regimens".

Post Placement (Head fixation)
During the pedestal implant surgery, or in a short procedure following pedestal implantation, we will cut small (~5-8 mm) incisions over each of the previously installed pedestals, and screw a pin into the pedestal that allows us to affix the animal to a head-holder. If necessary, we will add one or two sutures or skin glue to this installation to close the skin around the pin, but it is frequently not necessary. If performed as a minor procedure following pedestal implantation, the necessity of intravenous catheterization, fluids, and intubation will be determined in conjunction with the veterinary staff.

Craniotomy chamber placement
For this implantation we will follow the procedure outlined in [redacted]
After placing the animal in a stereotaxic frame, a parasagittal incision is made in the scalp exposing the cranium overlying the area of visual cortex. Using a pneumatic drill or trephine, a craniotomy is made in the skull. A craniotomy chamber is placed over the craniotomy. Small holes will be drilled for each of the legs of the chamber, and the chamber will be secured in position with bone screws sealing the craniotomy closed. The incision will be closed with suture, staples, or skin glue based on veterinary recommendation. Once the animal has awakened, normal post-surgical protocol will be followed per the SIG "Macaque Anesthesia/Analgesia/Antibiotic Regimens".

Implant Repairs
Occasionally implants may become loose, break, become chronically infected, or suffer from other possible conditions that make the appliance ineffective. In these cases the animals may undergo surgical procedures to either repair the device, replace it, or to remove it. These surgeries will always take place in consultation with the veterinary staff.

Implant Removal
Removal of the pedestals or implanted devices is performed using the same aseptic techniques and anesthetic methods used during implantation surgeries, unless the removal is done before euthanasia in a terminal procedure, in which case aseptic technique may not be utilized.

Vasectomy or Castration
Every attempt is made to transfer animals to a veterinary approved animal retirement facility after use. In some instances, it is necessary to vasectomize or castrate males so that they can be transferred to a retirement facility and housed with females. Sterilization procedures will be performed by ASU veterinary staff using procedures chosen at the discretion of the ASU veterinarian. Decisions regarding which surgery is to be performed will be made in consult with the ASU veterinarian and the receiving institution.

B. Anesthetic regimen:

The specific anesthetic regimen may vary based on the individual's needs, history, and temperament; it may include various combinations of the following medications as determined by the DACT veterinary staff.

Drug & concentration (e.g., mg/ml)	Dose (e.g., mg/kg) & maximum volume to be given	Route	Is this a DEA controlled substance (Y/N)?
Ketamine (100 mg/ml)	3-15mg/kg	IM	Y
Midazolam (5 mg/ml)	0.05-0.5 mg/kg	IM	Y
Tiletamine/Zolazepam (100 mg/ml)	1.5-10 mg/kg	IM	Y
Atropine (0.54 mg/ml)	0.02-0.05 mg/kg	IM	N
Glycopyrrolate (0.2 mg/ml)	0.005-0.01 mg/kg	IM	N
Sevoflurane	1-8%	Inhalation	N
Isoflurane	1-5%	Inhalation	N
Propofol (10 mg/ml)	2-5mg/kg (Bolus) 0.2-0.6 mg/kg/min (CRI)	IV	N

Please refer to the IACUC approved document "Macaque Anesthesia/Analgesia/Antibiotic Regimens"

Note: Use of gas anesthetics requires completion of the EH&S-based Anesthetic Gas Safety training prior to use and refreshed annually.

1. Describe measures used to indicate a surgical plane of anesthesia to keep animals from getting too light or too deep:
   Physiological status and anesthetic depth will be monitored by DACT veterinary personnel using parameters including reaction to stimuli, ECG, pulse-oximetry, end tidal gasses, heart rate, and ventilatory rate. Depth of anesthesia and vital parameter assessment and recording occurs approximately every 10 minutes and is adjusted as necessary based on these observations and measurements.

C. Additional pharmacological agents used during surgery (include analgesics, supportive medications, and research drugs):

Drug and concentration	Dose & max volume	Route	Purpose	Frequency	Is this a DEA controlled substance (Y/N)?
Betadine/Chlorhexidine	N/A	Topical	Disinfect surgical sites	Once, as needed	N
Bupivacaine	1-2 mg/kg	SC	Local anesthetic	Once, as needed	N
Cefazolin (330 mg/ml)	20-25 mg/kg	IV	Antibiotic	Every 4 hours, intraoperatively	N
Dexamethasone (2 mg/ml)	0.25-2 mg/kg	IM or IV	Reduce inflammation	As needed	N
Doxapram	2 mg/kg	Topical (tongue) or IV	Stimulate breathing	As needed based on vet assessment	N
Epinephrine	0.2-0.4 mg/kg	SC, IM or IV	Stimulate heart, vasoconstriction	As needed based on vet assessment	N
Gelfoam	Cut to size	Topical	Hemostasis/Seal surgical holes	Once, as needed	N
Hydromorphone (2 mg/ml)	0.05-0.2 mg/kg	SC, IM or IV	Analgesia	Once, PRN based on veterinary assessment	Y
Lactated Ringer's Solution	5-15 ml/kg/hr	IV	Fluid support	Continuous during surgery	N
Lidocaine containing gel/cream	Dab	Topical	Local anesthetic	Once	N
Mannitol (200 mg/ml)	0.25-2.2 g/kg over 20 minutes	IV	Reduce intracranial edema	As needed	N
Ophthalmic ointment	Dab	Topical	Prevent corneal desiccation	Once, as needed	N
0.9% NaCl Solution	5-15 ml/kg/hr	IV	Fluid support	Continuous during surgery	N

3% NaCl Solution	250 ml bolus over 30 minutes	IV	Reduce intracranial edema	As needed	N

D. Describe the steps taken to maintain an aseptic surgery:
Routine surgical steps include:
1. Disinfection of the exposed head and stereotactic mounting apparatus using alternate scrubs with alcohol and a disinfecting agent such as chlorhexidine or betadine. Sporocidin wipes are also used for certain stereotaxic components.
2. Standard scrubbing, sterile gowning and gloves, mask, bonnet/cap, and face shield are utilized by the surgeons.
3. Establishment of a sterile field using sterile drapes.
4. Use of tools and surgical instruments that have been either steam or gas sterilized.

E. What is the maximum duration of each surgery?
8 hours

F. Will any animals recover from surgery?
☐ No. This involves terminal, or non-survival, procedures; Appendix 2 is complete.
☒ Yes. Complete Section IV.

IV. POST-SURGICAL CARE

A. Is there a potential for post-operative pain or distress?
☐ No. Proceed to section C.
☒ Yes.

B. Will analgesics be used?
(For analgesic options, refer to the IACUC Standard Institutional Guideline on analgesia (https://researchintegrity.asu.edu/animals/procedures-library-and-guidelines) or contact a DACT veterinarian
☐ No. Provide a scientific justification:

☒ Yes. Complete the following.

Drug & concentration	Dose & max. volume	Route	Frequency	Is this a DEA controlled substance (Y/N)?
Buprenorphine (0.3 mg/ml)	0.01-0.03 mg/kg	IM or SC	Used PRN based on veterinary assessment	Y
Buprenorphine SR (1 mg/ml)	0.2 mg/kg	SC	Once, based on veterinary assessment	Y
Meloxicam (5 mg/ml injection; 1.5 mg/ml oral)	0.1-0.2 mg/kg	SC or PO	SID/variable duration based on procedure	N
Hydromorphone (2 mg/ml)	0.05-0.2 mg/kg	SC, IM or IV	PRN based on veterinary assessment	Y
Oxymorphone (1 mg/ml)	0.07-0.15 mg/kg	SC, IM or IV	PRN based on veterinary assessment	Y
Tramadol	1-2 mg/kg	PO	SID/BID variable duration and use based on procedure and	Y

			veterinary assessment	

Please refer to the IACUC approved document "Macaque Anesthesia/Analgesia/Antibiotic Regimens"

Who will administer these drugs?
DACT or trained PI staff

C. Post-operative routine care:

i. What other drugs will be administered, if any (e.g., antibiotics, fluids)?

Drug & concentration	Dose & max. volume	Route	Purpose	Frequency	Is this a DEA controlled substance (Y/N)?
Cefazolin (330 mg/ml)	20-25 mg/kg	IM	Antibiotic	BID/variable duration based on procedure	N
Cephalexin (50 mg/ml)	20-30 mg/kg	PO	Antibiotic	BID/variable duration based on the procedure	N
Enrofloxacin (22.7 mg pill or 22.7 mg/ml)	5 mg/kg	PO or IM	Antibiotic	SID/BID/variable based on the procedure	N

Please refer to the IACUC approved document "Macaque Anesthesia/Analgesia/Antibiotic Regimens" (choice of antibiotic and route of administration dictated by patient compliance. We try oral administration first, but default to injectable if NHP is not compliant)

ii. What other post-operative support and monitoring will be provided, how often, for how long, and by whom?
Pain assessment scoring is performed following major surgical procedures and continues until the pain score is 0 as determined by the veterinarians or trained research staff. Monitoring is provided by both trained DACT and PI personnel.

D. Is post-operative intensive care required?
☒ No. Proceed to section E.
☐ Yes.
What special care is required?

Who will provide special care and what are their qualifications?

For how long will special care be needed?

E. Will animals undergo multiple survival surgical procedures?
☐ No. Appendix 2 is complete.
☒ Yes. Describe which surgeries, the sequence (specifying time between surgeries), and frequency. Provide scientific justification:
See Appendix 2, section III for detailed descriptions of the surgeries. The sequence will begin with pedestal implantation. This allows time for osseointegration of the implanted parts to provide maximum security of the head-holding system. The second minor procedure allows us to implant posts to allow for head stabilization, and it may be performed in conjunction with pedestal implantation. After pedestal/post implantation, the animal is trained for several weeks. After the training is complete, a craniotomy is performed and the craniotomy chamber is implanted. Duration between surgeries may be variable but at minimum the animal will be allowed enough time to have healed and recovered from the previous surgery before proceeding to the following surgery in the sequence. Repair surgeries may also be performed to salvage an experiment or for the well-being of an animal after consultation with the veterinary staff. Finally, in preparation for retirement we may need to perform additional surgical procedures to remove the implants and vasectomize or castrate the animal.

IACUC Protocol Trackable Components Checklist

Protocol #: 21-1821R If for amendment, amendment #:

PI: [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 1/21/21

☐ No trackable components in this document

Exceptions to the Guide:

☒ Food/Fluid Regulation

Species: *Macaca mulatta*

What Restricted: Water

Parameters: Water will be available only at limited times during the day: first during the behavioral sessions and second at the end of the day. On days when animals are not working, their water allotment is split with one half administered in the AM and the second in the PM. Amounts of water provided will vary with the animal's weight, current work regime, and habits. This water restriction paradigm is used to provide an incentive for work. Details are found in the IACUC SIG "NHP Fluid Regulation".

☒ Prolonged Restraint

Species: *Macaca mulatta*

Details: Animals are restrained in primate chairs typically 2-8 hours per day, five to seven days per week. When performing tasks, the monkey also wears an aluminum halo that is affixed to the head by posts. The halo is then connected to an attachment that connects to the chair or the experimental setup table so the head cannot move. The head restraint is necessary in our tasks as the monkey's head must be perfectly still for recordings and to monitor eye position.

☒ Husbandry Deviation from the Guide

Species: *Macaca mulatta*

Deviation: Animals are pair housed except for recovery periods following surgery or if there is a danger of the paired animals injuring one another. Since we use such small number of animals, a suitable pairing partner may not be available.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)

Species: *Macaca mulatta*

Surgerie(s):

1) Implantation of pedestals for head fixation
2) Placement of posts for head fixation
3) Implantation of craniotomy chamber
4) Implant repairs
5) Implant removal
6) Vasectomy or Castration

IACUC Protocol Trackable Components Checklist

Multiple Major?: ☒ Yes ☐ No

☒ Hazardous Agents

Biological (list agent and hazard level):

Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.): 10% Formalin ± 20% glycerin (Toxin)

Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.): MRI (magnetic fields and up to ~110 dB noise), CT scan and radiographs (X-ray radiation)

☐ Non-Centralized Animal Housing

Location:

Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Date: 10.21.2021

ARIZONA STATE UNIVERSITY
IACUC ANNUAL REVIEW

I. Currently approved protocol

Protocol Number: 21-1821R
Protocol Title: Vision Restoration Using Magnetic Stimulation of Visual Cortex
Principal Investigator: [redacted]
☒Funded ☐Unfunded

II. Status of Project

A. Were the animal activities conducted?

i. ☒ **Yes, they were conducted.** If yes,

1. Were there any significant animal welfare issues (morbidity or mortality, complications, etc.) encountered over the past 12 months?
   a. ☐ Yes. Describe (include the problem, approximate number of animals affected, and resolution).

   b. ☒ No. Proceed to item II B.
2. Were all unanticipated welfare issues reported?
   a. ☐ Yes. Proceed to item II B.
   b. ☐ No. Describe. Proceed to item II B when completed.

ii. ☐ **No, they were not conducted.** If the protocol will be terminated, complete the Final Review form.

1. If the protocol will remain active, why were animal activities not conducted?

Proceed to Section II B.

B. Have there been any recent findings, either from this study or a related study that would change the planned use of animals?

- Species Used
- Animal Numbers
- Procedures
- Criteria to Measure/Monitor Pain or Distress
- Alternatives to Painful Procedures
- Restraint
- Amelioration and Control of Painful Procedures
- Estimation of Potential Postoperative/Intervention Pain
- Preoperative/Postoperative/Chronic Care
- Euthanasia/Disposition of Animals
- Animal Care and/or Use Sites

i. ☐ Yes. Complete a separate Request for Changes form describing all proposed changes as well as the scientific rationale for these changes. Proceed to item III.

ii. ☒ No. Proceed to item III.

III. Updated Information

A. Did the pain status stated on the protocol remain appropriate for the procedures performed?

i. ☒ Yes. Proceed to item III B.
ii. ☐ No. If no, please describe: Proceed to item III B when completed.

B. Has there been new funding added to the project?

i. ☐ Yes. Provide new grant(s) information:
Granting Agency:
Title:
ASU Proposal or Award number:

☒ No.

IV. Progress Report (for research or teaching protocols only)

Provide a statement on progress under this protocol over the past 12 months. Include any presentations or publications that have resulted from this protocol during the past 12 months.

NHP task training began in February of 2021. We began stimulation in May, after chamber placement surgery. Data collection is ongoing. The NHP works 4-5 days per week, with stimulation occurring 2-3 times per week depending on NHP temperament and cooperation.

Surgical Procedures:
02/18/21 - Pedestal/post placement
05/18/21 Craniotomy and placement of cranial chamber

V. Personnel

All personnel who work with animals are required to have animal care training within the last four years. ASU IACUC training modules can be completed at https://asu.co1.qualtrics.com/jfe/form/SV_b2b2XRXRRs1309f. Personnel are required to have Level III training certification on file with the IACUC office in order to perform procedures independently (without supervision). See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

*** Procedures other than husbandry, handling, or behavioral testing MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel who are not Level III certified are supervised at all times.**

A. List the names, titles, affiliations, and roles of **ALL** persons currently involved in the research or teaching activity.

Name	Title	ASURITE name	Role in Protocol: What activities will each person perform on live animals ONLY while under direct supervision?	Role in Protocol: What activities will each person be allowed to perform **independently** (including appropriate Level 3 certification*) at the time of protocol submission?	Species with which individual will have direct contact ("none, "all", or list species)	FOR IACUC USE ONLY Training Confirmation

				Responsible for overall conduct of all studies, including performing surgeries, poling, handling, restraint, implant maintenance, and training.		1/2022 OHSP
[redacted]	PI	[redacted]			Macaca mulatta	
	Lab Coordinator		Assistance with surgical procedures	Lab management, running behavioral tasks, poling, handling, restraint, implant maintenance, and training.	Macaca mulatta	11/2018 OHSP
	Assistant Research Professor			Primary data collection, poling, handling, restraint, training, perform surgeries, implant maintenance	Macaca mulatta	11/2018 OHSP

B. If any of the above listed personnel are new to the protocol, describe their years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

C. List the names of any individuals no longer involved with the research (these individuals will be removed from the protocol and DACT will be notified):

VI. Certification

By signing this report, I certify that, to the best of my knowledge, the information included herein is accurate and complete. I understand that continued animal use past the scheduled termination date of the protocol requires IACUC approval. I also understand that should the animal use under this protocol require ANY change from that stated in the protocol, prior approval by the IACUC is required.

[redacted]

Principal Investigator's Signature

10.21.21
Date

FOR IACUC USE ONLY
Annual Review Determination

ANNUAL REVIEW APPROVAL SIGNATURES:

DocuSigned by:
[redacted]
Chair, IACUC (or Designee) — January 27, 2022 — Date

[redacted]
Attending Veterinarian (or Designee) — January 27, 2022 — Date

DocuSigned by:
[redacted]
IACUC Member — January 27, 2022 — Date