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

Animal Protocol Review

ASU Protocol Number: 21-1818R
Protocol Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network
Principal Investigator: [redacted]
Date of Action: 12/21/2020

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. Contact [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:

Total # of Animals: 6
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/21/2020 – 12/20/2023

Sponsor: National Science Foundation
ASU Proposal/Award #: [redacted]
Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network

Signature: [redacted] Date: 12/30/2020
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

IACUC Use Only	**IACUC Protocol #:** 21-1818R
Date: 10/30/2020	☐ 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: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network

SPECIES REQUESTED: Rhesus macaque, *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: National Science Foundation Deadline: 08/31/2021

Co-Investigator(s):

Proposal Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network

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 #: 18-1630R

☐ 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, as well as providing enrichment, surgical assistance and pre/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.

Although the posterior parietal cortex (PPC) has long been considered an important area for multisensory integration, spatial awareness, and spatial attention, recent studies point to an additional and very major role for this area in movement planning. One important advance has been the finding that there is an anatomical map of intentions within the PPC, with different areas specialized for different behaviors. We will be examining the interrelationship between some of the parietal areas involved in arm movement planning and control. In particular, we will be studying whether area 5 and area 7b of the PPC are involved in the integration of visual and somatic information regarding arm position. We will also be studying whether neurons in these areas are involved in joint manipulative actions such as object handovers. The information gained by this project will provide insight into the role of the PPC to producing and monitoring movements, which will help identify better treatments for individuals with damage to this area of the brain.

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.**

Our research is aimed at understanding how the brain combines sensory information from various sources and uses it to guide our limb movements. Such basic sensorimotor functions can be altered by 'strokes' and traumatic brain injuries, as well as by the normal aging process and developmental abnormalities. We have been studying these functions by monitoring the activity of cells in a brain region known to be involved in sensorimotor control, the posterior parietal cortex (PPC). In the period covered by the previous protocol, we examined the interaction of visual and somatic signals in the superior parietal lobule (SPL) as monkeys reached to and actively maintained their arm position at multiple locations in a frontal plane. On half of the trials both visual and non-visual feedback of the endpoint of the arm was available, while on the other trials visual feedback was withheld. When examined individually, many neurons were tuned to arm position but relatively few neurons were modulated by the presence/absence of visual feedback. However, decoding of limb position at the population level was generally more accurate when visual feedback was provided. These findings support a multimodal representation of limb position in the SPL but suggest that visual signals regarding limb endpoint position are only

weakly represented in this area, and only at the population level. The relatively weak responses to visual feedback about limb position in the PPC suggest that the locus of integration of visual and somatic information may reside in other cortical areas. As a result, for the current protocol we intend to record in other parts of the parietal cortex (i.e. areas MIP and 7b) as well as areas of the frontal lobe, specifically the dorsal premotor cortex (PMd) and the ventral premotor cortex (PMv). Since the integration of sensory and motor information is known to be highly context-dependent, we will also be exploring the integration of visual and somatic information in a different task involving the handing over and receiving of objects. This will initially involve handovers between an animal and a human participant but will eventually involve handovers between the animals and a robot.

We expect to use up to 6 male animals weighing between 3-17 kg in this study, with two carried over from our previous protocol, please refer to section C. 3. for justification. Each animal will receive chronically implanted devices over multiple possible surgeries, including pedestals for head fixation, cortical recording electrodes, and chambers for driving electrodes through the dura in both hemispheres (see Appendix 2 Surgical Procedures). All animals used under this protocol will be housed and tested in a purpose-built primate research area within the vivarium. Each primate has its own home cage and may share a play space with additional conspecifics. All animals are allowed time for contact, play, grooming, etc., except in the intervals immediately after implant surgeries and those under an IACUC approved pairing exemption. The animals are purchased in small groups to limit problems with territorial dominance and social isolation while in quarantine. 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 is consulted on designing routine care, and who are always available in the case of unusual circumstances (wounds, infections, etc.). The general overall timeline of events from acquisition to training is shown in the table below:

Estimated Duration (months)	Action
2	Quarantine
2	Pole/collar training and pedestal implant
1	Chair training
12 to 36 (per study)	Behavioral training/recording

The timelines above reflect the estimated duration of events; actual duration may vary. For example, NHPs may participate in multiple studies, which would increase their time training/recording by more than the 36 months stated.

The disposition of an animal following its participation in a project will depend upon a variety of factors. Three options would include: an IACUC-approved transfer of the animal to another project with different implant requirements, euthanize and perfuse the animal and process the brain for histological analysis, or retire the animal to an ASU approved sanctuary. These decisions will be made on a case-by-case basis in consultation with the DACT veterinarians and IACUC. Some of the factors considered in the decision include: (1) what other projects might require an animal; (2) is the animal suitable for another project; (3) status of the animal‘s condition; and (4) status of any chronic arrays the animal has implanted.

1. Behavioral Tasks

Animals are water regulated and perform tasks for fluid reward. For all tasks, the animal is placed comfortably into a NHP chair, the chair is placed into the behavioral apparatus, the head is restrained in a head-holder and the behavioral task is started. At the end of each successful trial, the apparatus delivers a drop of fluid to the animal. The animals typically work for 2 to 3 hours per session; however, sessions may last up to 6 hours. If an animal stops working before consuming an amount of fluid that is adequate to meet its hydration needs, the animals will be given a break of 5-15 minutes. During the breaks, the animal will remain seated in the setup. Treats may be given during this time. If the animal will not continue working after this break, a second 5-15 minutes will be given. If the animal does not continue working after this second break a third and final break of 5-15 minutes will be given. If the animal does not commence working after the third break, the animal will be returned to its cage for the day. At the end of the day, the animal will receive a fluid supplement to bring its total water consumption for each day to a level adequate to meet the animal’s hydration needs. See the IACUC approved Standard Institutional Guideline (SIG) “NHP Fluid Regulation”.

1.1 Visual Fixation Task

The fundamental behavioral task employed in our experiments is an unrestrained point-to-point arm movement to

an array of targets in a 3D space. However, because parietal neurons are modulated by eye movements as well as arm movements, these reaching movements must be performed while an animal holds its eyes still. Thus, the animal is initially trained to look at and maintain fixation upon a visual stimulus presented in a virtual reality environment (VR). The animal's eye movements are monitored using a commercially available optical remote eye tracking system. To get a fluid reward, the monkey moves its eyes to fixate the target ball, then maintains fixation until the end of the trial.

1.2 3D Reaching Task

The point-to-point arm movements are also carried out in a VR environment. The NHP chair is placed in a VR frame in which the monkey views virtual images in a mirror. The mirror is suspended in front of its face at a 45 degree angle, and the animal's arm is free to move on the opposite side of the mirror. The animal cannot see its real arm, and there will be no physical targets for it to touch. Instead, it will see virtual targets (balls floating in 3D space), a fixation ball, and a cursor ball representing its hand position in space. Variations in this model will also include a 3D modeled arm resembling that of the individual animal completing the task. To get a fluid reward, the monkey moves the cursor ball into a target ball by moving its real hand in space, while simultaneously gazing at the fixation ball. In the VR task, the virtual targets can be placed anywhere within the monkey's reachable workspace. Reward procedures and training schedules are as described for the Behavioral Task in part 1.

1.3 Target Perturbation Task

This task is a variant of the 3D Reaching Task. Here, a virtual target for the movement will be presented then, once the animal begins to move to this target, its location will be changed to another location in the workspace, requiring a movement correction. All other procedures are as described for the 3D Reaching Task.

1.4 Learning Tasks

The PPC is thought to play an important role in motor learning, thus in addition to the motor tasks described above we will also be developing learning tasks. These tasks will involve changing the association between the animal's felt limb position and its visually perceived position. This will be accomplished by displacing the cursor ball from the true position of the limb by a small amount. Studies in humans have shown that subjects initially make reaching errors as a result of such perturbations, but then adapt their movements accordingly. We will be examining how that adaptation process manifests in the PPC. Aside from the visual perturbation, the physical parameters of the task will be identical to the 3D Reaching Task, as will the reward procedures and training schedules.

1.5 Joint Action (Handover) Tasks

First, a chaired monkey will be trained to receive food and non-food objects, such as different shapes or textured items, from a human agent during handover tasks involving different arm configurations through a plexiglass sheet placed between the human and monkey. The hole in the plexiglass will be approximately 2" in diameter to limit the monkey's reach through the plexiglass sheet. A 2nd plexglass barrier will prevent the monkey from ingesting the non-food objects but still allow for the receiving of a liquid reward through a tube inserted through the barrier.

Handover variations learned from the first step will be used to program a robot arm for the next step. In one set of trials, neural activity will be recorded as animals simply observe the robot delivering an object to each of several handover positions ("observation"). Neural activity will then be recorded as animals subsequently reach out to receive the object from the robot at these positions ("execution"). The combined response to observation and execution will be compared to activity recorded during a second set of trials where the robot and monkey perform the handover in unison ("joint action"), which requires the precise coordinated timing movements between agents that is a hallmark of joint action. Finally, for the third step, the data collected in these tasks will be used to implement a neurally-controlled handover task, in which the animal's neural activity controls the movements of the robotic arm to perform the handover task.

2. Cranial Implants

2.1 Head holders

The procedures described below require that the animal's head be securely fixed to avoid movements while the electrodes are in the cortex. To accomplish this, we have modified a commercially available system to suit our needs. The head holder consists of a halo that is securely attached to the NHP chair. Attachment of the animal to the halo is accomplished by means of three posts which protrude through the skin. These posts are mounted on pedestals which are attached to the animal's skull with bone screws. The pedestals are placed at least six weeks prior to implantation of the chamber (see Appendix 2 Surgical Procedures).

When implanting the posts, small incisions are made over each pedestal, and a post is threaded into a hole located on top of the pedestal. This post provides the mechanism by which the head is secured to the halo.

2.2 Fixed Cortical Recording Electrodes

The fixed cortical recording electrodes consist of arrays of microwires or silicon-substrate microprobes. The wire electrodes are made from materials such as stainless steel, tungsten, or platinum-iridium with an insulative coating of Teflon, Parylene-C, or the equivalent. The diameters of these wires may range from about 20 microns to 70 microns. The electrodes may be further coated with special polymers or biological proteins to facilitate cell adhesion and minimize the natural foreign-body response of the brain. Arrays may be purchased from commercial vendors (e.g. [redacted] or provided by a research group at the [redacted]

Standard microwire arrays are composed of 16 microwires arranged into a 2 x 8 array with 250um spacing. This occupies 1.75 mm^2 of surface area and driven to a depth of ~10 mm, which displaces about 0.704 mm^3 of brain tissue (16 wires at 4.42 x 10-3 mm^2 surface area per wire). We generally implant 4 of these devices, which occupies a total of 7 mm^2 surface area, and at a depth of 10 mm displaces about of 3 mm^3 of brain. We will not exceed this total displacement by recording electrodes without prior approval by the Attending Veterinarian and the IACUC.

Each of the wires on our microwire arrays provides one recording site. Alternative technologies such as the silicon-substrate Michigan probe and polyimide-substrate electrodes being developed in our department can provide multiple recording sites per wire, thus providing a much higher recording-site displaced-brain ratio. These refinements reduce the implant profile and increase the number of neurons we are able to record. As these technologies develop, we may to use them to record neural activity. If that is the case, we will submit an amendment prior to using these electrodes.

Refer to Appendix 2 Surgical Procedures for details.

2.3 Recording of Parietal Activity with Movable Electrodes

We will record the activity of neurons in the PPC using movable electrodes that are driven through the dura using a standard microdrive [redacted] The microdrive attaches to an 18 mm cylindrical recording chamber that is mounted directly onto the animal's skull.

Activity will be recorded extracellularly with commercially available varnish-coated tungsten or platinum-iridium microelectrodes [redacted]

2.4 Surgical Implantation of Cortical Electrodes and Recording Chamber

Surgery will be performed under aseptic conditions with general anesthesia. The surgeries will take place in the dedicated surgical suite in the vivarium. Refer to Surgery Appendix 2 for details.

In order to design and decide on proper placement of the recording chambers and the electrode arrays, we may obtain MRI and/or CT images of each monkey. Generally, there will be one trip per NHP for each procedure; additional images may be taken for verification purposes.

Please refer to the IACUC approved SIG "NHP Imaging" for details.

3. Daily Protocols

3.1 Behavioral Training and Water Regulation

During behavioral training, each animal will spend up to 6 hours per day in the laboratory. There will be a training period before the surgery of approximately six months, but this varies based on the difficulty of the task. At the conclusion of the initial recording period, another surgery may be performed over the contralateral hemisphere and the animal will perform the task with its other arm for approximately the same period.

The animals will be maintained on a regulated water schedule in order to motivate them to perform the behavioral task according to the IACUC approved Standard Institutional Guideline (SIG) “NHP Fluid Regulation”.

Prior to implantation of recording devices, the animals will work up to 5 days per week to train them to perform the behavioral tasks. Our simplest task, the 3D center out task, typically takes 2-3 months to train the animals to an adequate performance level (600-1000 trials per day). Our more complex tasks, the target perturbation and learning tasks, can take up to 6 months to train. Once the animals have been trained to criterion performance and implanted with recording devices, they may begin working up to 7 days per week to acquire data. This will continue for as long as the animal is producing significant data, typically between 6 months and 3 years.

If the animal is to be implanted on the opposite hemisphere, there will be a shorter retraining interval in which the animal learns to perform the task with the opposite arm. Typically, this is between one and three months. After the animal reaches criterion performance on the new side, the animal will be re-implanted and recording/working schedules of up to 7 days per week will resume.

During each recording session, the animal will be comfortably seated in a standard NHP chair that restricts body and head movements. Most sessions will last 2-3 hours, with special attention to the animal’s comfort when its head is fixed. The head fixation system will be carefully adjusted if the animal shows signs of distress. If the distress cannot be relieved, the animal will be removed from the chair and the health of the implants checked prior to re-restraining the animal. If the implants appear healthy (no infection, no loose fixtures), then in the subsequent sessions the animal will be lightly tranquilized with acepromazine by the PI or DACT veterinary staff (starting with the lowest dose) before affixing the head. This practice will continue to allow the animal to slowly adapt to having its head secured. This practice is rarely done, since acclimation to the halo is started early. The NHPs are generally comfortable having their head secured once recording sessions begin. Once the animal has been seated in the chair, the halo will be affixed to the chair for the duration of the recording session.

If a recording chamber is implanted, to start each recording day, we will open up the acute recording chamber, remove any fluid that had accumulated in the chamber, and then rinse the inside of the chamber with a dilute antiseptic solution followed by sterile saline. We will then attach a hydraulically driven microdrive onto the implanted cylindrical recording chamber. A sterile standard metal recording electrode is attached to the microdrive and slowly moved down to and then through the dura. The electrode will be advanced through the dura and into the cortex in order to record neurons. If an electrode is to be used multiple times it will be cleaned and sanitized between sessions in a sonicator bath filled with dilute chlorhexidine. The electrode will then be rinsed with sterile saline before being used.

At the end of the recording session, the electrode will be withdrawn, and the microdrive detached from the chamber. While the chamber is open, we will again cleanse the inside of the chamber with dilute antiseptic solutions (refer to the IACUC SIG “NHP Implant Maintenance”). We may also treat the inside of the chamber with sterile white petroleum jelly (applied with a sterile tip applicator) to reduce scarring on the surface of the dura [redacted]. Finally, after extensive rinsing with sterile saline, we will seal the chamber with a bead of petroleum jelly and replace the cap to keep the dura moist.

As needed, we will carefully remove accumulated scar tissue from the dura in the chamber. Scar tissue will be removed either in the awake NHP seated in the NHP chair using topical 0.25% bupivacaine or, if extensive debridement is needed, under anesthesia. Care of the dura and interior of the recording chamber will be routinely assessed in consultation with the DACT veterinarians. If fixed cortical arrays are implanted, after the monkey’s head is restrained, the screw cap that protects the connector is removed and the connector is plugged in to the recording system. The margins of the implant are cleaned in accordance with the IACUC SIG “NHP Implant Maintenance”.

4. Terminal Measures

4.1 Micro-lesion and Histology

One or two weeks prior to the perfusion, small lesions will be electrolytically induced around the ends of a sample of the electrodes. These lesions will be produced by passing 10 mAmps of current through the electrode for five to ten seconds. This procedure will be performed while the animal is awake but these lesions will not cause pain to the animals and we do not expect such small lesions to produce neurological signs or symptoms. However, animals will be closely monitored for signs of discomfort and stress. If any neurological signs or symptoms are observed the procedure will be stopped and the animal will be sedated before the procedure is continued. We will then use standard histological techniques to reconstruct the recording positions of the electrode in the cerebral cortex, ensuring that we recorded neural activity from the proper structure. In addition, detailed anatomical measurements will be made of the arms so that an accurate biomechanical model can be constructed to satisfy the specific aim of determining muscle force during the task.

If not retired to a sanctuary following the study, the animals will be euthanized at the end of the experiment using the following protocol: the animal is first sedated with an appropriate sedative (e.g., ketamine). 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 (a phosphate buffer solution), 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.

5. References

[redacted]

6. Animals carried over from previous protocol:

[redacted] Currently working under tasks 1.1-1.4. This animal is water restricted and has pedestal/post implants. He is currently performing trained tasks.

[redacted] Currently working under tasks 1.1-1.4. This animal is water restricted and has pedestal/post currently performing trained tasks.

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?
   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 also employ computer simulation and modeling techniques in this study, such methods cannot substitute for actual observations. Tissue cultures of cortex are not a possible alternative because connections to the eyes and other cortical areas would be lost. It would be impossible to study the responses of cortical neurons to complex visual displays or their modulations by eye movements commanded by the animal. Brain-slice preparations, it should be noted, require surgery and anesthesia for the removal and transfer of the tissue to a regulated diffusion chamber for
   study. 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?
   Unlike rodents or birds, NHPs are capable of and can be trained to perform behavioral tasks involving the processing of complex visual and auditory stimuli and motor behavior. This species has been selected

because their neural processes and physiology closely resembles that of humans. Rhesus macaques have binocular, color vision and hear sounds in the same range as humans. They have similar eye and reach movements and hand-eye coordination as humans. The skull size is large enough to accommodate the recording chambers and arrays used (a smaller NHP species would be unsuitable for this reason). Old world primates of the family Cercopithecidae (of which the Rhesus monkey is a member) have a brain structure that is very similar in anatomy to that of humans more so than other nonhuman primate species. Also, there is considerable reference material on the anatomy of the rhesus macaque brain available in the scientific literature. The [redacted] is just one example of the current publications available.

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.
   Recordings are required from many neurons in more than one animal in order to establish the validity of the results. A sample size of two to three animals per study is the acceptable standard in the field and is dictated by individual variation in each animal and by the number of cells needed in a study. We anticipate a maximum of two studies per year, thus, we may use up to 5 animals in a year that will be carried forward into year 2 and year 3. We emphasize that 6 NHPs over three years is an absolute maximum, and anticipate that further refinement of our methods will increase the amount and quality of data obtained from each animal.

4. What refinements, if any, have been made to reduce the number of animals used and the potential detrimental effects on the study animals?
   To reduce the number of animals used we will characterize the response of neurons in multiple areas (areas 5, and 7b of the PPC; areas PMd and PMv of the frontal lobe) using the same set of animals (rather than one set of animals for area 5 and another for area 7b for example). In addition, for the parietal recordings we will attempt to use the same recording chamber to characterize the two areas in each animal.

   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 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. We are using state of the art technology to minimize the invasiveness of procedures including using a minimally invasive halo head restraint system instead of the commonly used larger acrylic head-cap, smaller cortical connectors that are biocompatible and do not require the need for dental acrylic to stabilize. We make every attempt to pair house animals, as well as provide daily environmental enrichment.

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

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*): 10/25/2020
Database(s) used: PubMed, AWIC
Publication years covered by the search: 1900-2020
Keyword combinations used: parietal cortex AND arm AND multisensory integration AND primate; premotor cortex AND arm AND multisensory integration AND primate

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*): 10/25/2020
Database(s) used: PubMed, AWIC
Publication years covered by the search: 1900-2020
Keyword combinations used: parietal cortex AND arm AND movement AND primate; non-human primates/monkeys AND neurophysiology AND alternatives; non-human primates/monkeys AND water restriction AND alternatives; non-human primates/monkeys AND head restraint AND alternatives; non-human primates/monkeys AND craniotomy AND alternatives

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.

Searches for alternatives to head restraint and craniotomy in non-human primates/monkeys produced no relevant results. That is, although publications were returned by these searches these publications did not discuss alternatives to the procedures that we are currently using in the lab.

Searches for alternatives to fluid restriction produced one relevant result [redacted] reported the results of an experiment probing the use of social stimuli (images of conspecifics) as rewards for four rhesus macaques performing simple cognitive tasks. Based on their findings, they could not recommend the use of social stimuli, in this form, as a refinement to current fluid restriction protocols.

References:

[redacted]

D. Describe any other procedures (e.g., participation in meetings, review of journals) that are used to explore and evaluate alternatives: Attendance at large international meetings covering general neuroscience (e.g. Society for

Neuroscience annual meeting) and small international meetings focused on motor control systems neuroscience (e.g. Neural Control of Movement and Computational and Systems Neuroscience meetings), personal communications with other investigators in the field, and routine reading of recent journal papers.

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			6		6

*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] 10/25/2020

Principal Investigator - Print — Date

[redacted] 10/25/2020

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
	PI			PI; perform surgeries, poling, handling, restraint, training	Macaca mulatta	9/2020 Basics 11/2018 NHP OHSP
	Associate Professor			Surgical assistance	Macaca mulatta	9/2020 OHSP
	Lab Coordinator		Surgical assistance, data collection	Lab management, poling, handling, training, restraint, implant maintenance	Macaca mulatta	11/2018 OHSP
	Graduate Assistant		Surgical assistance	Poling, handling, restraint, implant maintenance, data collection	Macaca mulatta	2/2019 OHSP
	Research Scientist		Surgical assistance	Perform surgeries, poling, handling, restraint, training, data collection, implant maintenance	Macaca mulatta	3/2019 OHSP
	Undergraduate Student		Assist with data collection, poling, handling, data collection, implant maintenance		Macaca mulatta	10/2018 OHSP

[redacted]	Neurosurgeon/Adjunct Faculty		Perform surgical procedures on anesthetized animals only		Macaca mulatta	Visiting surgeon - OHSP only done when needed

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:
All have completed IACUC Level I and II NHP training modules. [redacted] has nearly 24 years' experience with handling, training and performing cranial implant surgeries in NHPs. [redacted] has almost 30 years' experience performing NHP cranial surgeries. [redacted] has more than 12 years of NHP experience, including 6 years handling and training rhesus macaques. [redacted] has approximately 5 years' experience handling, training, and assisting with rhesus macaque surgeries. [redacted] has approximately 7 years' experience working with NHPs. [redacted] has been working in the lab for almost two years. She works with NHPs under the supervision of Level 3 lab personnel.

[redacted] specializes in neurosurgery at [redacted]. He will only be working with anesthetized animals.

The Lab Coordinator, PI, or a Level 3 graduate student will provide any necessary handling training. Students do not work unsupervised with any NHP until they have received Level 3 Handling Certification. PI and DACT veterinary staff provide any necessary surgical training. No surgical procedures are performed without the supervision of either a PI or DACT veterinary staff.

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: 6

C. Sex: M Age or Weight Range: 3-17kg

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

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 when animals are done working. On days when animals are not working, their water allotment is split AM and PM. Amounts of water provided will vary with the animal's weight, current work regimen, 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.
The animals will be seated in an NHP chair during behavioral testing for a maximum of 6 hours, up to 7 days a week, but usually 5. During this time the monkey will be performing the task(s) described above. They will be given rewards based on performance. Breaks will be given as needed, based on performance. 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 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 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.

In order to prevent the NHP from accessing implanted devices or gloves for data acquisition, an arm restraint may be used to limit the use of one arm. The arm restraint consists of a Velcro wrist cuff and nylon tether, which is attached to the work table. This setup allows the animal some movement of the restrained arm, but not enough to reach/interfere with sensors on the opposite arm. This will only be used while the monkey is performing a task. A primate jacket, shirt, sleeve, and/or glove (Lomir Biomedical, or lab-constructed) may also be used to position small LED sensors down the length of one arm so that arm position may be tracked. The animal will be gradually habituated to any restraint device prior to its full use.

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. 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.

For monkeys with open craniotomies, we may use a surgical grade silicone elastomer as a chamber plug. This has 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. We propose to use this method in two ways: (1) in new craniotomies where the monkey is not yet fully trained and (2) in monkeys that are currently recording. 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. Prior to placing a plug in a monkey that currently has a chamber, we will first debride all of the granulation tissue present. If an infection occurs, we propose that the plug will not be used until treatment of the infection is complete.

Reference for silicone elastomer- http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2291023/

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 may not be pair housed during recovery after surgical procedures, about 2-4 weeks. They will be pair housed after consultation with the veterinary staff. 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?

The animal is identified by their ID # which is tattooed on their chest or inguinal area. Animals either have the tattoo upon arrival or are tattooed by DACT staff during quarantine exams. 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
Cyanoacrylic glue (e.g., [redacted]	As needed	Extradural	Close/seal dura mater	Once, 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-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	0.07-0.15 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
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
Sevoflurane	1-8%	Inhalation	Anesthetic	Continuous, during surgery	Y	N
Tiletamine/Zolazepam	1.5-10 mg/kg	IM	Anesthetic	As needed	Y	Y
Tramadol	1-2 mg/kg	PO	Analgesic	SID-BID, 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:
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. Herpes B virus is not being used in animals, but can be transmitted to personnel if there is an NHP bite/exposure.

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, or 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 "NHP 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?

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

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.

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 such as hydromorphone to prevent any pain felt by the sternotomy. The NHP is then deeply anesthetized with anesthetic gas. 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.

We do not anticipate that the work under this protocol will require euthanasia, however, if we experience certain unplanned events or issues such as implant failure, abnormal morphology that we consider study related, etc, we may decide to euthanize to investigate the cause.

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
Hydromorphone	0.05-0.2 mg/kg	IM	Y	Used in coordination with perfusion
Phosphate 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. Placement of pedestals for head fixation
2. Placement of posts for head fixation
3. Implantation of recording chamber/cannulas (right and left hemispheres)
4. Implantation of chronic arrays (right and left hemispheres)
5. Explant of chamber/arrays
6. Implant repairs
7. Vasectomy or Castration

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

II. PRE-SURGICAL CARE

B. 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.

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):
Each animal will undergo several surgeries. We have found that patient and well-structured implantation of each of the devices necessary for these experiments provides for better longevity of appliances and more extensive data. A maximum of six types of devices may be surgically implanted onto each animal (listed above items 1-4). In order to design and decide on proper placement of the electrode arrays, we may obtain MRI and CT images of each monkey, if available. 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.

Pedestals and post surgeries will always be performed first, but the other surgeries may occur in different orders. If chamber attachments and array implantations are done during the same surgery, they will be considered separate procedures in terms of the described limit. Additional repair surgeries may be required to correct problems (e.g., loose or broken pedestal replaced in a similar location, loose acrylic cap). These repair surgeries will be discussed and performed in consultation with the DACT veterinarians. Any additional experimental surgeries, but limited to the seven surgery types described above (e.g., replace a malfunctioning electrode, move the location of existing pedestals to new locations) will be submitted to the IACUC Chair and Attending Veterinarian for approval. 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.

Preoperative Care and Induction:
The day before surgery the animal is fasted overnight to prevent vomiting 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 "NHP 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 surgical site 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] o[redacted] in addition to surgical skin glue unless otherwise directed by the veterinarian based on the circumstances.

1. Pedestal Implants
This procedure provides mounting points for three pins that are eventually installed to affix the animal's head. The pedestals are small (1.5 cm) tripods that are affixed flush with the skull using bone cortex screws. For each pedestal, a 2 cm incision is made over the selected site (while the animal is positioned in a stereotax), and the skin and muscle layers progressively dissected to the skull. The area that will support the pedestal is then scraped with a periosteal elevator, and the pedestal 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 three legs of the pedestal, and the pedestal 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 "NHP Anesthesia/Analgesia/Antibiotic Regimens".

2. Posts
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.

3. Chamber Implant
This procedure is to provide a mount for attaching microelectrode drive units and a port through the skull that allows us to drive electrodes through the dura and into the brain. In this procedure, we identify the location of the chamber, and then make an incision that will allow the remaining skin to close tightly around the external portion of the chamber. We then carefully remove underlying tissue and scrape the bone around the site of the implant with a periosteal elevator. We will then create an 18 mm circular defect with a hand trephine, mount the chamber over the defect using 4-6 cranial screws, and secure the base of the chamber with dental acrylic. If necessary, the incision will be closed in layers around the implant site.

4. Electrode array implant
In this procedure we implant an array of microelectrodes subdurally into the cortex for chronic recording. In this procedure, with the NHP in a stereotax, we will open a large area of the scalp, typically with the skin preserved and reflected backwards. Once we have access to the bone, a section of bone no larger than 25 mm in diameter will be cut out using a surgical drill and preserved. We will then dissect a dural flap overlying the target implant site, and reflect the dura back. When the cortex is exposed, we will then implant the microelectrode array by placing it onto the cortex and pressing it into position. The dura will then be closed over the implant using surgical grade cyanoacrylic glue (e.g. [redacted] or equivalent product), and the piece of bone placed back into the defect. If the piece of bone is large (10 to 25 mm), it will be secured in position with orthopedic straps and screws. We have previously had IACUC approval for the use of expired [redacted] s only approved for an 18-month shelf life, with the expiration date guaranteeing the sterility/integrity of the packaging, the ability to gel, and the time it takes to gel. The gel is purchased in packs of 5 that cost over $6000. Given the infrequency of our NHP surgeries, we do not use up the entire product before expiration. Thus, an already expensive product gets cost prohibitive to the point of it being essentially unavailable. Thus, we tested the efficacy of a vial of [redacted] that expired 08/02/2010 and was over 4 years expired. Since its sterility was questionable, the expired pack was gas sterilized. It was then mixed together per instructions and it gelled within the guaranteed 3.5 seconds. Based on these results, we would like to be able to use [redacted] that has passed its expiration date by up to 12 months, which is far shorter that the expiration age of the vial we tested. Th[redacted] will be re-sterilized before use. If the defect is smaller than 10 mm, the bone

section may be secured in position by packing the exposed gaps with [redacted] and covering the entire defect with a thin layer of acrylic cranioplasty. The wires and connector will be routed to a convenient location on the skull and affixed to the skull with a combination of bone screws and dental acrylic. Finally, the overlying tissue will be closed in layers (similar suture and skin glue as used for pedestals or chambers) over the implant site and around the exposed connector.

5. 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.

6. 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.

7. 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. Vasectomies/orchiectomies will be performed by ASU veterinary staff using procedures chosen at the discretion of the ASU veterinarian.

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 "NHP 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
Cyanoacrylic glue (e.g., DuraGen/DuraSeal)	As needed	Extradural	Close/seal dura mater	Once, as needed	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:

- Disinfection of the exposed head and stereotactic mounting apparatus using alternate scrubs with alcohol and a disinfecting agent such as chlorhexidine or betadine. Sporicidin wipes are also used for certain stereotaxic components.

- Standard scrubbing, sterile gowning and gloves, mask, bonnet/cap, and face shield are utilized by the surgeons.

- Establishment of a sterile field using sterile drapes.

- 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 a e ment	Y
Buprenorphine SR (1mg/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 ba ed on veterinary assessment	Y
Oxymorphone (1 mg/ml)	0.07-0.15 mg/kg	SC, IM or IV	PRN based on veterinary a e ment	Y
Tramadol	1 2 mg/kg	PO	SID/BID variable duration and use based on procedure and veterinary assessment	Y

Please refer to the IACUC approved document "NHP 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 "NHP 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 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 section II.A 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. The third and fourth surgeries involve implantation of systems that allow us to record the activity of multiple neurons simultaneously. In some of our experiments, we need to drive individual electrodes and record the activity of separate individual neurons from one day to the next. The array surgeries involve implantation of systems that allow us to record the activity of multiple neurons simultaneously and obtain stable recordings over long periods from the same sets of neurons. In addition, because each hemisphere controls movement in only one side of the body, we may duplicate surgeries on the left and right side in order to maximize our data acquisition from a single animal. 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 order to prepare animals for retirement, we will need to perform additional surgical procedures to remove the implants and vasectomize or castrate the animal.

IACUC Protocol Trackable Components Checklist

Protocol #: 21-1818R If for amendment, amendment #:

PI: [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 12/9/20

☐ 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 when animals are done working. On days when animals are not working, their water allotment is split between the AM and PM. Amounts of water provided will vary with the animal's weight, current work regimen, 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: The animals will be seated in an NHP chair during behavioral testing for a maximum of 6 hours, up to 7 days a 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. In order to prevent the animals from accessing implanted devices or gloves for data acquisition, an arm restraint may be used to limit the use of one arm. The arm restraint consists of a Velcro wrist cuff and nylon tether, which is attached to the work table. Arm restraint will only be used while the monkey is performing a task. A primate jacket, shirt, sleeve, and/or glove may also be used to position small LED sensors down the length of one arm so that arm position may be tracked.

☒ Husbandry Deviation from the Guide

Species: *Macaca mulatta*

Deviation: Animals may not be pair housed during recovery after surgical procedures (~2-4 weeks). Suitable pairing partners may not be available for all animals.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)

Species: *Macaca mulatta*

Surgerie(s): A maximum of 6 types of devices may be surgically implanted per animal. Procedures include:

1. Placement of pedestals for head fixation
2. Placement of posts for head fixation
3. Implant of recording chamber/cannulas (right and left hemispheres)
4. Implant of chronic arrays (right and left hemispheres)

IACUC Protocol Trackable Components Checklist

5. Explant of chamber/arrays
6. Implant repairs
7. Vasectomy or Castration

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

From: Karen Kibler
To: [redacted]
Cc: IACUC@asu.edu
Subject: December protocols
Date: Monday, December 21, 2020 4:08:37 PM
Attachments: [redacted]21-1818R Final.docx[redacted]

Hi [redacted]

I've attached the [redacted] protocol (approved as presented), the [redacted] protocol (approved as modified), and the [redacted] protocol (DR – I have made the change now and am approving it). The [redacted] protocol is still in the DR process.

Thanks,
Karen

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

[redacted]

Animal Protocol Review

ASU Protocol Number: 21-1818R RFC 1
Protocol Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network
Principal Investigator: [redacted]
Date of Action: 8/10/2021

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

The request for changes was administratively approved to ad [redacted] as additional personnel to the protocol.

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 Research Support Services group within 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 DAC[redacted] prior to starting surgeries.
☐ Other requirements:

Total # of Animals: 6
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/21/2020 – 12/20/2023

Sponsor: National Science Foundation
ASU Proposal/Award #: [redacted]
Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network

Signature: [redacted] Date: 8/12/2021
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

ARIZONA STATE UNIVERSITY

Institutional Animal Care and Use Committee

REQUEST FOR CHANGES TO AN APPROVED PROTOCOL

Protocol No. 21-1818R RFC 1
Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network
Principal Investigator: [redacted] Email Address: [redacted]
If not PI, who should we contact for questions related to this amendment: [redacted] Email Address: [redacted]

☒ Funded ☐ Unfunded

Requested Change (check all that apply):

☐ New procedures to be performed – complete Part A, and Appendix 1 and/or 2 as applicable, and sign assurance.
☐ New species and or an increase in the number of animals to be used complete Part A and sign assurance.
☐ New location of housing or procedures complete Part A and sign assurance.
☒ New personnel complete Part B and sign assurance.
☐ Other (includes changes in dosages, funding, etc.) complete Part A and sign assurance.

A. Description of Requested Changes

For new procedures or additional animals that are USDA-covered species (all mammals EXCEPT mice and rats bred for research), list the **Category of Pain:**

For new procedures or additional animals that are not USDA-covered species, will there be the potential to involve more than slight or momentary pain or distress that will NOT be relieved with anesthetics, analgesics, tranquilizer drugs, or other methods for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

If you are adding a procedure that could create pain or distress, you need to include a **literature search** for alternatives.
If you are adding a new survival surgery, submit a surgical checklist.
If you are requesting an increase in animal numbers, provide justification with supportive statistics.
If you are adding additional funding sources, provide the grant agency, grant title and ASU proposal or award number.

Describe the changes you are requesting.

B. Addition of 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). Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. 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.**

Name	Title	ASURITE name	What activities will each person perform on live animals ONLY while under direct supervision?	What activities will each person be allowed to perform independently (including appropriate Level 3 certification*)	Species with which individual will have direct contact ("all" or list species) *	IACUC USE ONLY Training (mm/yy)

				at the time of protocol submission?		
[redacted]	Student	[redacted]	None. [redacted] will be working in the computer lab only, so no direct contact with the NHPs will be necessary.		None	8/2021 OHSP not applicable

For each individual, describe the individual's training and 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:

At this time [redacted] will be working in the computer lab only, assisting with data analysis and running the monkey task work. If we anticipate additional interaction with the NHPs, we'll amend the protocol at that time.

Assurance

As Principal Investigator of this protocol, I assure that all procedures will be conducted as described in this request for changes and that personnel will receive appropriate additional training prior to conducting any new procedures that are not listed above.

SIGNED: [redacted]

Principal Investigator _08.06.2021_ Date

For IACUC use only:

☒ Administratively approved - Approving administrator: [redacted] Date of approval: 8/10/2021

☐ Administratively handled by VCV - Veterinarian providing verification: Date of verification:

Sources used for verification:

☐ Approved by Designated Review – Designated reviewer: Date of approval:

☐ Approved by Full Committee Review Primary reviewer: Date of approval

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

Animal Protocol Review

ASU Protocol Number: 21-1818R RFC 2
Protocol Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network
ASU Principal Investigator: [redacted]
Date of Action: 11/23/2021

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

Request for changes was approved to add [redacted] additional personnel.

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 Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contact [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 DAC [redacted], prior to starting surgeries.
☒ Other requirements: IBC approval is required

Total # of Animals: 6
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/21/2020 – 12/20/2023

Sponsor: National Science Foundation
ASU Proposal/Award #: [redacted]
Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network

Signature [redacted] Date: 11/23/2021
IACUC Chair or ***Designee***
Cc: IACUC Office, IACUC Chair

PERSONNEL MODIFICATION FORM
IACUC and IBC

This form can be used to add or remove participants from both an IACUC protocol and an IBC disclosure. Please submit only one form to Research.Integrity@asu.edu and it will be processed by both committees.

Principal Investigator Nam [redacted]	Phone:
Dept: [redacted]	Email [redacted]

Participant #1	Add to: ☒ IBC [redacted] ☒ IACUC #21-1818R Delete from: ☐ IBC # ☐ IACUC #	FOR ORIA USE ONLY Training Ver f cat on
Name [redacted]	ASURITE: N/A Email: [redacted]	
Project Responsibilities in IBC: Perform surgical procedures on anesthetized animals only		
Experience/Training in These Responsibilities: [redacted] has ~15 years of NHP research experience working in labs a [redacted] He has also helped perform NHP surgeries with collaborators at the [redacted] and [redacted] He has performed approximately 30 NHP surgeries including about 10 chronic multi-electrode array implants. (also see attached CV)		Visiting surgeon – training not required
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: [redacted] will be performing the microelectrode array implant surgery (as described in Appendix II Section III Item A4 of this protocol). This will occur in consort with/under the supervision o [redacted]		
Species: Macaca mulatta Experience and training with species and procedures: [redacted] has performed approximately 30 NHP surgeries including about 10 chronic multi-electrode array implants. (also see attached CV)		

Participant #2	Add to: ☐ IBC # ☐ IACUC # Delete from: ☐ IBC # ☐ IACUC #	FOR ORIA USE ONLY Training Ver f cat on
Name:	ASURITE: Email:	
Project Responsibilities in IBC:		
Experience/Training in These Responsibilities:		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision:		
Species: Experience and training with species and procedures:		

Assurance

As Principal Investigator, I assure that personnel will receive appropriate training prior to working with animals or biological materials as applicable.

Principal Investigator Signature [redacted] Date: 11.18.2021

FOR ORIA USE ONLY	☐ IBC Approved	☒ IACUC Approved 11/23/21

Revised 11/20/12

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

Animal Protocol Review

ASU Protocol Number: 21-1818R RFC 3
Protocol Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network
Principal Investigator: [redacted]
Date of Action: 12/1/2021

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

The request for changes was approved by Designated Review to add an alternative dural repair material to the protocol.

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 Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contact [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:

Total # of Animals: 6
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/21/2020 – 12/20/2023

Sponsor: National Science Foundation
ASU Proposal/Award #: [redacted]
Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network

Signature: [redacted] Date: 12/1/2021
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

ARIZONA STATE UNIVERSITY

Institutional Animal Care and Use Committee

REQUEST FOR CHANGES TO AN APPROVED PROTOCOL

Protocol No. 21-1818R RFC 3
Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network
Principal Investigator: [redacted] Email Address [redacted]
If not PI, whom should we contact for questions related to this amendment: [redacted] Email Address: [redacted]

☒ Funded ☐ Unfunded

Requested Change (check all that apply):

☐ New procedures to be performed – complete Part A, and Appendix 1 and/or 2 as applicable, and sign assurance.
☐ New species and or an increase in the number of animals to be used complete Part A and sign assurance.
☐ New location of housing or procedures complete Part A and sign assurance.
☐ New personnel complete Part B and sign assurance.
☒ Other (includes changes in dosages, funding, etc.) complete Part A and sign assurance.

A. Description of Requested Changes

For new procedures or additional animals that are USDA-covered species (all mammals EXCEPT mice and rats bred for research), list the **Category of Pain:**

For new procedures or additional animals that are not USDA-covered species, will there be the potential to involve more than slight or momentary pain or distress that will NOT be relieved with anesthetics, analgesics, tranquilizer drugs, or other methods for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

If you are adding a procedure that could create pain or distress, you need to include a **literature search** for alternatives.
If you are adding a new survival surgery, submit a surgical checklist.
If you are requesting an increase in animal numbers, provide justification with supportive statistics.
If you are adding additional funding sources, provide the grant agency, grant title and ASU proposal or award number.

Describe the changes you are requesting. During array implant procedures, dural repair material is necessary to minimize the risk of cerebrospinal fluid leakage. The materials which we currently have approval to use require a lengthy lead time, and are not currently available. In lieu of those, we would like to add an alternative to the protocol. This alternative, called [redacted] is very similar to [redacted] It can be cut to fit the needed space, and will effectively seal the edges of the dura mater.

[redacted]

B. Addition of 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). Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. 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.**

Name	itle	ASURIT	What activ ties will each person pe form on live	What activities will each person be	Species with which individual will have	ACUC

		name	animals **ONLY** while under direct supervision?	allowed to perform **independently** (including appropriate Level 3 certification*) at the time of protocol submission?	**direct contact ("all" or list species) ***	**USE ONLY Training (mm/yy)**

For each individual, describe the individual's training and 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:

At this time, [redacted] will be working in the computer lab only, assisting with data analysis and running the monkey task work. If we anticipate additional interaction with the NHPs, we'll amend the protocol at that time.

Assurance

As Principal Investigator of this protocol, I assure that all procedures will be conducted as described in this request for changes and that personnel will receive appropriate additional training prior to conducting any new procedures that are not listed above.

SIGNED: [redacted]

Principal [redacted] 11.29.2021 Date

For IACUC use only:

- ☐ Administratively approved - Approving administrator: Date of approval:
- ☐ Administratively handled by VCV - Veterinarian providing verification: Date of verification:
  Sources used for verification:
- ☒ Approved by Designated Review – Designated reviewer: Karen Kibler Date of approval: 12/1/2021
- ☐ Approved by Full Committee Review – Primary reviewer: Date of approval

From: Karen Kibler
To: [redacted]
Cc: iacuc@asu.edu
Subject: RE: [redacted]21-1818R RFC 3 - Ready for Assignment
Date: Monday, November 29, 2021 12:23:11 PM
Attachments: image001.png

Hi, [redacted]

Please send for DR process approval with me as the DR. If the process is approved, I approve as written.

Thanks,
Karen

From [redacted]
Sent: Monday, November 29, 2021 12:07 PM
To: Karen Kibler [redacted]
Cc: iacuc@asu.edu
Subject [redacted]21 1818R RFC 3 Ready for Assignment
Importance: High

Hello Karen,

The attached RFC has cleared vet review as written and is ready for assignment. This is the RFC that [redacted] emailed about last week. They are hoping to secure approval as soon as possible for a procedure scheduled on 12/2.

Sincerely,

[redacted] Compliance Coordinator, Office of Research Integrity & Assurance
Arizona State University | Knowledge Enterprise | Operations
[redacted] f 480-965-777
[redacted] | http://researchintegrity.asu.edu
How am I doing? Email m[redacted] or send a Sun Award
Chat with me on Teams! (ASU Users Only)

[redacted]

To: [redacted]acuc@asu.edu
Subject: RE: URGENT Action Required: Designated Review fo[redacted]1 1818R RFC 3

From [redacted]
Sent: Monday, November 29, 2021 12:45 PM
To: [redacted] Dale DeNardo [redacted] Karen Kibler [redacted]
Cc: iacuc@asu.edu
Subject: URGENT Action Required: Designated Review fo[redacted]21-1818R RFC 3
Importance: High

Designated Reviewer: Karen Kibler
Principal Investigator: [redacted]
Peer Reviewer: N/A
Protocol Number: 21-1818R RFC 3

Tracking:	Recipient	Response
	[redacted]	Yes: 11/30/2021 5:09 PM
		Yes: 11/29/2021 12:48 PM
		Yes: 11/29/2021 12:47 PM
	Dale DeNardo [redacted]	Yes: 11/29/2021 3:19 PM
		Yes: 11/30/2021 7:17 AM
	Karen Kibler [redacted]	Yes: 11/29/2021 12:23 PM
		Yes: 11/29/2021 12:46 PM
		Yes: 11/29/2021 1:37 PM
		Yes: 11/29/2021 4:57 PM

This request is time-sensitive. Please respond ASAP.

A request for an amendment to the referenced protocols has been submitted. The request for amendment is attached and the various related protocols are available at the SharePoint site. The Designated Review process allows IACUC members, by voting, to permit the Designated Reviewer to approve or disapprove an amendment request.

Select "**YES**" if you approve the use of the designated review process for this amendment.

Select "**NO**" if you disapprove the use of designated review process regarding this amendment request. The amendment will then be reviewed at the next monthly IACUC meeting.

Select "**Abstain**" if you would like to abstain from the vote for any reason.

Select "**Recuse**" if you have a conflict of interest.

Please indicate your approval or disapproval of the request for designated review by using the YES or NO button in the toolbar at the top of this message. The use of "YES or NO" buttons allows you to submit comments along with your choice. **You may also send your comments to me directly or to the primary reviewer without using the selection buttons. Please copy me on all correspondence and email related to this request.**

Sincerely,

IACUC Protocol Trackable Components Checklist

Protocol #: 21-1818R If for amendment, amendment #: 3

PI: [redacted]

Species: NHP Highest Category of Pain: D

Completed by: Dale DeNardo Date completed: 11/29/21

☐ 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 when animals are done working. On days when animals are not working, their water allotment is split between the AM and PM. Amounts of water provided will vary with the animal's weight, current work regimen, 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: The animals will be seated in an NHP chair during behavioral testing for a maximum of 6 hours, up to 7 days a 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. In order to prevent the animals from accessing implanted devices or gloves for data acquisition, an arm restraint may be used to limit the use of one arm. The arm restraint consists of a Velcro wrist cuff and nylon tether, which is attached to the work table. Arm restraint will only be used while the monkey is performing a task. A primate jacket, shirt, sleeve, and/or glove may also be used to position small LED sensors down the length of one arm so that arm position may be tracked.

☒ Husbandry Deviation from the Guide

Species: *Macaca mulatta*

Deviation: Animals may not be pair housed during recovery after surgical procedures (~2-4 weeks). Suitable pairing partners may not be available for all animals.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)

Species: *Macaca mulatta*

Surgerie(s): A maximum of 6 types of devices may be surgically implanted per animal. Procedures include:

1. Placement of pedestals for head fixation
2. Placement of posts for head fixation
3. Implant of recording chamber/cannulas (right and left hemispheres)
4. Implant of chronic arrays (right and left hemispheres)

IACUC Protocol Trackable Components Checklist

5. Explant of chamber/arrays
6. Implant repairs
7. Vasectomy or Castration

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: 09.08.2021

ARIZONA STATE UNIVERSITY
IACUC ANNUAL REVIEW

I. Currently approved protocol

Protocol Number: 21-1818R
Protocol Title: Multimodal State Estimation through Neural Coherence in the Parieto-Frontal Network
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.

Multi-session Analysis of Movement Variability While Reaching in a Virtual Environment

The acquisition of neurophysiological data during awake, behaving animal experiments typically involves experimental sessions lasting several days to weeks. Therefore, it is important to understand natural fluctuations in behavioral performance over such periods. Here we quantified patterns of movement variability for reaches performed by two monkeys across five daily experimental sessions. The monkeys were trained to move in an immersive virtual reality (VR) environment that was designed to resemble the experimental room. Visual feedback of the limb was provided using VR avatar arms that were controlled through a reflective marker-based motion capture system. Additionally, tactile cues were provided in the form of physical reach targets. Spatial variability was characterized at early (peak acceleration) and late (movement endpoint) kinematic landmarks. We found that the magnitude of variability was generally larger at peak acceleration than at the endpoint but was relatively consistent across days and within animals. The spatial characteristics of variability were also generally highly consistent at peak acceleration both within and between animals but were noticeably less so at the endpoint. The results highlight the benefits of using early kinematic landmarks such as peak acceleration for quantifying movement variability in reaching studies involving animals.

[redacted]

Effects of Tactile Cues on Arm Movement Planning and Execution in a Virtual Environment

During the planning of arm movements, visual and somatosensory inputs are combined to compute an estimate of the current arm position through a process known as multisensory integration. This estimate is used to determine the desired movement vectors as well as joint-based motor commands for action. Although multisensory integration for reaching has been extensively studied in humans, investigations involving non-human primates (NHP), which serve as an important model for understanding the underlying neural mechanisms, are relatively sparse. Here, we used a semi-immersive virtual reality (VR) environment paired with a motion capture system to study the integration of visual, proprioceptive, and tactile cues during reaching movements. Two NHPs were trained to wear a motion capture sleeve on their right arm, which served to animate a virtual replica of their own limb in real-time within the VR environment. The VR arm and environment were projected from a 3D monitor onto a mirror blocking vision of the animals' actual arm. After acquiring a central starting position, the animals made reaches to one of four targets in the horizontal plane during which visual feedback of the virtual arm was provided or removed on a trial-by trial basis. Physical properties of the virtual start and target positions were rendered to resemble those of real tactile cues, which were circular plastic

buttons that were added or removed from the workspace in separate blocks of trials. Analysis focused on quantifying the variability in hand movement direction and distance (relative to the starting position) as a function of movement extent (i.e., from 10-100% of movement on a given trial, in increments of 10%). We found that for all task conditions, directional variability decreased with movement extent, while conversely there was an increase in variability in distance traveled. Variability in direction and distance was reduced in conditions where tactile cues were provided, though this effect was more apparent during the later stages of the reaching movements. Reduced variability in direction and distance was also observed in conditions employing visual feedback but only when tactile cues were absent. These results show that the presence of tactile cues reduces the variability of reaching movements performed in a virtual environment. Moreover, the results suggest that tactile cues interact in a complex manner with ongoing visual and proprioceptive feedback to determine motor output.

[redacted]

Reaching Errors Resulting from the Misestimation of Arm Position

The planning of visually-guided reaching movements requires the integration of visual and somatosensory inputs to construct an estimate of the arm's position. In some contexts (e.g., in dimly lit environments, during experimental manipulation of sensory feedback, or following neurological damage) the integration of visual and somatosensory inputs can lead to an arm position estimate that is biased and imprecise, leading to errors in movement planning and execution. We recently developed an experimental paradigm to study the neural correlates of visual, proprioceptive, and tactile integration for arm position estimation in non-human primates. In this paradigm, animals are trained to reach from a defined start position to four targets in a semi-immersive VR environment that incorporates a fully-rendered monkey avatar arm. On half of the trials, the avatar arm appears in a position consistent with the physical location of the starting position, and on the other trials the position of the viewed arm is shifted several centimeters away from the physical start location. In addition, physical start locations and their corresponding shifted arm positions are varied in separate blocks of trials. Behavioral analyses focus on changes in initial movement directions that are induced by shifting the arm position. However, although some behavioral and simulation studies have characterized the effects of arm position perturbations, the results that are reported are highly specific to the employed experimental paradigm. Here we used computer simulations to predict the behavioral consequences of shifting the viewed location of the arm in the context of our own experimental paradigm. Two joint (shoulder and elbow) arm movements in the horizontal plane were simulated. An inverse model was used to determine the joint torques that corresponded to nominal endpoint trajectories and a forward model was then used to simulate these movements in an open-loop fashion, i.e. feedback control was not included. Perturbations were simulated by introducing uncompensated bias and variance into the estimate of initial hand position. The simulations produced movement errors that were both movement direction- start position- and shift-dependent. For the primary start position used in our experiment, simulation results were generally consistent with behavioral data obtained from one animal. These results provide important insights into the movement errors that result from misestimating arm position, which could prove useful for understanding corresponding errors that arise during investigations of the neural correlates of multisensory integration, as well as those arising from neurological damage.

[redacted]

Trimodal integration of visual, tactile, and proprioceptive cues influences reach planning in a virtual environment

The planning of reaching movements requires integration of visual and proprioceptive cues to estimate the position of the arm before and during movement. Although bimodal (visuo-proprioceptive) integration has been well-characterized in human subjects, less is known about trimodal integration processes, e.g., for reaching movements performed when tactile information is initially present. Moreover, few studies have examined multisensory integration in non-human primates (NHP), which are more amenable to examination of the neural correlates of multisensory integration. Here, we used a semi-immersive virtual reality (VR) environment to study

how visual, proprioceptive, and tactile cues about arm position are integrated prior to movement execution. A NHP was trained to reach from a

single start position to four targets in VR while viewing a fully-rendered monkey avatar arm that was animated via a reflective-marker based motion capture system. Trials began with no visual feedback of the arm but once the starting position was cued and acquired, the avatar arm was displayed for 1-1.5 seconds prior to movement. The virtual arm appeared either in a position consistent with the physical location of the starting position (veridical vision (VV)), or shifted 6 cm toward the animal's body (perturbed vision (PV)). VV and PV conditions were varied on a trial-by-trial basis. In addition, tactile feedback was varied in separate blocks of trials by adding or removing a circular plastic button from the starting position. Evidence for multisensory integration was assessed by comparing mean reach directions at peak tangential acceleration across visual and tactile conditions. We observed target-dependent differences in initial reach directions between PV and VV conditions, regardless of tactile conditions. For targets with clear differences, initial reach directions tended to be more accurate (i.e. less deviated from cued target directions) on VV trials than PV trials. Notably, both the target-dependence and the direction of deviation on PV trials were largely consistent with forward dynamics simulations of visually perturbed reaches. Initial reach directions also tended to be more accurate for reaches made with tactile feedback than without, with larger effects being observed for targets influenced by arm visual feedback. The results indicate that visual and tactile cues influence the planning of reaches performed in a virtual environment. The consistent nature of the observed effects suggest visual and tactile cues are integrated along with proprioception to form a more accurate estimate of initial arm position used in movement planning.

[redacted]

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
[redacted]	PI	[redacted]		PI; perform surgeries, poling, handling, restraint, training	Macaca mulatta	9/2020 Basics 11/2018 NHP OHSP
	Associate Professor			Surgical assistance	Macaca mulatta	12/2021 Basics 9/2020 NHP OHSP

[redacted]	Lab Coordinator	[redacted]	Surgical assistance, data collection	Lab management, poling, handling, training restraint, implant maintenance	Macaca mulatta	11/2018 OHSP
	Graduate Assistant		Surgical assistance	Poling, handling, restraint, implant maintenance, data collection	Macaca mulatta	2/2019 OHSP
	Undergraduate Student		Assist with data collection, poling, handling, implant maintenance		Macaca mulatta	10/2018
[redacted]	Undergraduate Student		None. [redacted] will be working in the computer lab only, so no direct contact with NHPs will be necessary.		None	8/2021 OHSP not applicable
	Neurosurgeon		Perform surgical procedures on anesthetized animals only		Macaca mulatta	Visiting surgeon, training not applicable 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): [redacted]

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

09.08.2021
Date

FOR IACUC USE ONLY
Annual Review Determination

ANNUAL REVIEW APPROVAL SIGNATURES:

DocuSigned by:
[redacted]
Chair, IACUC (or Designee)

December 16, 2021
Date

DocuSigned by:
[redacted]
Attending Veterinarian (or Designee)

December 16, 2021
Date

DocuSigned by:
[redacted]
IACUC Member

December 16, 2021
Date

From: [redacted]
To:
Cc: IACUC@asu.edu
Subject: [redacted]
Date: Wednesday, June 22, 2022 8:41:00 AM
Attachments: image001.png
Importance: High

Hello Dr [redacted]

[redacted] has been removed from your IACUC protocol 21 1818R due to an expired OHSP clearance. They are not allowed to work with animals until they have renewed their OHSP clearance and have been added back to the protocol.

Thank you,

[redacted] MLS | Compliance Coordinator, Office of Research Integrity & Assurance
Arizona State University | Knowledge Enterprise | Operations
[redacted] | http://researchintegrity.asu.edu
How am I doing? Email my [redacted] or send a Sun Award
Chat with me on Teams! (ASU Users Only)