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

[redacted]

Animal Protocol Review

ASU Protocol Number: 20-1743R
Protocol Title: TENPO – transdermal neuromodulatior
Principal Investigator: [redacted]
Date of Action: 9/26/2019

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

The protocol was approved.

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 independently. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training.

Total # of Animals: 4
Species: NHP **Pain Category: D**

Protocol Approval Period: 9/26/2019 – 9/25/2022

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signatur[redacted] Date: 10/7/2019
IACUC Chair or *Designee*

Cc: IACUC Office
IACUC Chair

IACUC Use Only	**IACUC Protocol #:** 20-1743R
Date: 9/4/2019	☒ IBC ☐ RSC ☒ Chem

ANIMAL USE PROTOCOL
ARIZONA STATE UNIVERSITY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
(Revised March 2019)

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: TENPO – transdermal neuromodulation

SPECIES REQUESTED: *Macaca mulatta*

I. PERSONNEL INFORMATION

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

NAME: [redacted] TITLE: Associate Professor

AFFILIATION: School of Biological and Health Systems Engineering Office Phone # [redacted]

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

B. Additional contact, if any, for IACUC business

NAME: [redacted] TITLE: Laboratory Coordinator

AFFILIATION: School of Biological and Health Systems Engineering Office Phone # [redacted]

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

C. Protocol Type

☐ Non-funded research

☐ Internal Funding

Account Number:

☒ External Funding (Grant/Contract)

Granting Agency: [redacted] Deadline:

Co-Investigator(s): [redacted]

Proposal Title: [redacted]

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 #: 17-1538R

☐ 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.

The ascending reticular activating system (RAS) is a collection of nuclei and circuits that sort, filter, integrate and transmit incoming sensory information from the brainstem to the cortex to regulate sleep/wake cycles, arousal/alertness, attention, and sensorimotor behaviors. RAS networks distribute information from the sensory environment to the cortex and are capable of rapidly triggering neurobehavioral transitions across different states of behavioral awareness and consciousness. We propose to develop a neural interface capable of dynamically and electrically modulating the RAS networks in order to provide a chemical-free approach to optimizing normal human performance. Our ultimate objective is to develop protocols that implement transdermal electrical neuromodulation (TEN) to improve the performance of human subjects on visual, sensorimotor, and cognitive tasks that are critical for National Security and U.S. Department of Defense (DoD) Mission Readiness. Here we will develop, evaluate, and deliver training protocols that incorporate TEN of the trigeminal nerve and other cranial nerves (facial and vagal for example) to study their effects on non-human primates.

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.**

Task 1: TENPO Development

We will use a multi-pronged approach to developing transdermal electrical neuromodulation for performance optimization (TENPO) for the nonhuman primates, with two overall goals. First, to develop the methods to test our non-invasive stimulation techniques in the nonhuman primate (NHP) where in addition to assessing behavioral and performance effects of our stimulation, we can also measure the release of neuromodulators from structures like the locus coeruleus (LC), the nucleus accumbens (NAc), and the nucleus basalis, and perhaps more importantly for understanding the mechanisms of any effects we observe, we can also measure TENPO-induced

changes in cortex. Secondarily, we can begin to narrow down the parameter space we need to explore in applying TENPO to human subjects. While many devices have been conceived and taken to market that drive transdermal stimulation of the trigeminal nerve (see Figure 1), little is known about the exact course of action of these systems in humans. Here we plan to use the NHP as a model for the impact of these stimulation methods, but in this case even less is known about the distribution of current to the trigeminal nerves and their central targets. In this task we will develop that knowledge through a series of quantitative measures and models.

For this task, we will require two NHPs. We will first implant cuff electrodes on one or more branches of the trigeminal nerve in the face. Standard cuff electrodes will be employed. The first implantations will be carried out by a plastic surgeon familiar with the technology, who will show us his techniques (and has assisted on other primate surgeries on our protocols). Connectors for these electrodes will be routed under the scalp to the location of the cortical recording chamber: this chamber is designed for the introduction of a cannula to direct the Fast Scan Cyclic Voltammetry (FSCV) probe. A cannula with a stop will be used to insert the electrodes to the desired depth to record in the structures of interest. During the procedure, each of the three main branches of the trigeminal nerve, the ophthalmic, maxillary, and mandibular, will be stimulated in turn while neuromodulator release is measured from the LC. Each selected branch will be gently exposed, and a stimulating electrode placed onto the nerve. The nerve will then be stimulated at 50 Hz for 0.5-1 second every 5 seconds for 1 minute while norepinephrine (NE) release is measured in the LC using FSCV. The port will remain secured to the skull to continue measuring these neuromodulators.

In lieu of cuff electrodes, we may choose to stimulate via surface electrodes. We will perform surface electrode stimulation and record electrical activity in the brain (from the chamber implant procedure) prior to the cuff electrode implant. As surface electrode stimulation is completely noninvasive, we may attempt this method first in order to potentially avoid having to surgically implant cuff electrodes. We will first place surface electrodes over one or more of the facial foramen through which the branches of the trigeminal nerve exit the cranium (supraorbital, infraorbital, and/or mental). The facial foramen and nerves will be located from previously obtained MRI/CT data. The NHP will be awake with their head restrained in their primate chair. They will be fully acclimated to calmly sitting in the primate chair before we attempt this procedure. Their face will be shaved at the site of electrode placement, and standard stimulating surface electrodes will be employed. During the procedure, each of the three main branches of the trigeminal nerve, the ophthalmic (above the brow), maxillary (on the cheek next to the nose), and mandibular (on the lower jaw aligned with the canine tooth), will be stimulated in turn (one, two, or all three may be stimulated at any given time) while electrophysiological activity is measured from the locus coeruleus. The stimulation parameters of current intensity, frequency, number of pulses, and pulse width will be modulated individually (in a manner similar to that in Hulsey et al., 2017) to identify the combination of individual parameters that maximally drives activity in the LC. As the parameter with the greatest implications in regard to safety, current intensity will range from 0-100 mA, which is consistent with literature guidelines [redacted] and commercial products currently on the market [redacted]

In order to most efficiently collect data, we may use other areas of the body surface for stimulation including the shoulder, back, chest, or legs. In any individual session, one or two of these locations will be selected. Two locations may eventually be selected so that we may make a direct comparison of response to stimulation, e.g., of the

maxillary branch of the trigeminal nerve and the dermatome of C8. In most cases, however, only one site will be stimulated, requiring placement of two electrodes. Our main purpose in this funding is to compare the responses in LC elicited from stimulation of the ophthalmic, maxillary, and mandibular branches of the trigeminal nerve, and these three locations will continue to be our focus.

The NHP will be awake with their head restrained in a primate chair, a process that the animal will be acclimated to beforehand. The site of electrode placement will be shaved as necessary to ensure proper skin contact for the employed standard stimulating surface electrodes. This will be approximately every two weeks and only use an electronic clipper with a guard, as electrode gel will be used to mitigate any issues with stubble/short growth. The NHPs are acclimated to this device and procedure to ensure cleanliness and accessibility of chambers and head restraint posts. During the sessions, the targeted area will be stimulated while electrophysiological activity is measured from the locus coeruleus.

Once we collect and analyze the data from the preliminary experiment, we will then measure the baseline release of NE and dopamine (DA) from the LC and NAc, respectively, as the animal sits in a primate chair, and is exposed to the TENPO waveforms. The animal will be acclimated to the primate chair and transdermal electrical neuromodulation (TEN) stimulation prior to the experiment. We will choose stimulation parameters and waveforms that are substantially equivalent to existing pulsed current neurostimulation devices that are indicated for use on the body, head and face and intended for over-the-counter (OTC) and prescription use (see Figure 1 for examples). We will design TEN waveforms, which have output characteristics within the range of already existing devices to preserve safe approaches that are substantially equivalent to existing devices indicated for various purposes. FSCV measures will be taken during stimulation, followed by multiple daily measurements over the first two days (not to exceed 6 hours in the restraint chair), and daily measurements for several days. In the first instances, measures will be more frequent and extend over a longer period after the stimulation, until we have established baseline expectations for how long any change in release is likely to last. In later phases of the experiment we hope to be able to focus neurochemical measurements over more critical phases of the experiment, which may reduce the number of measurements.

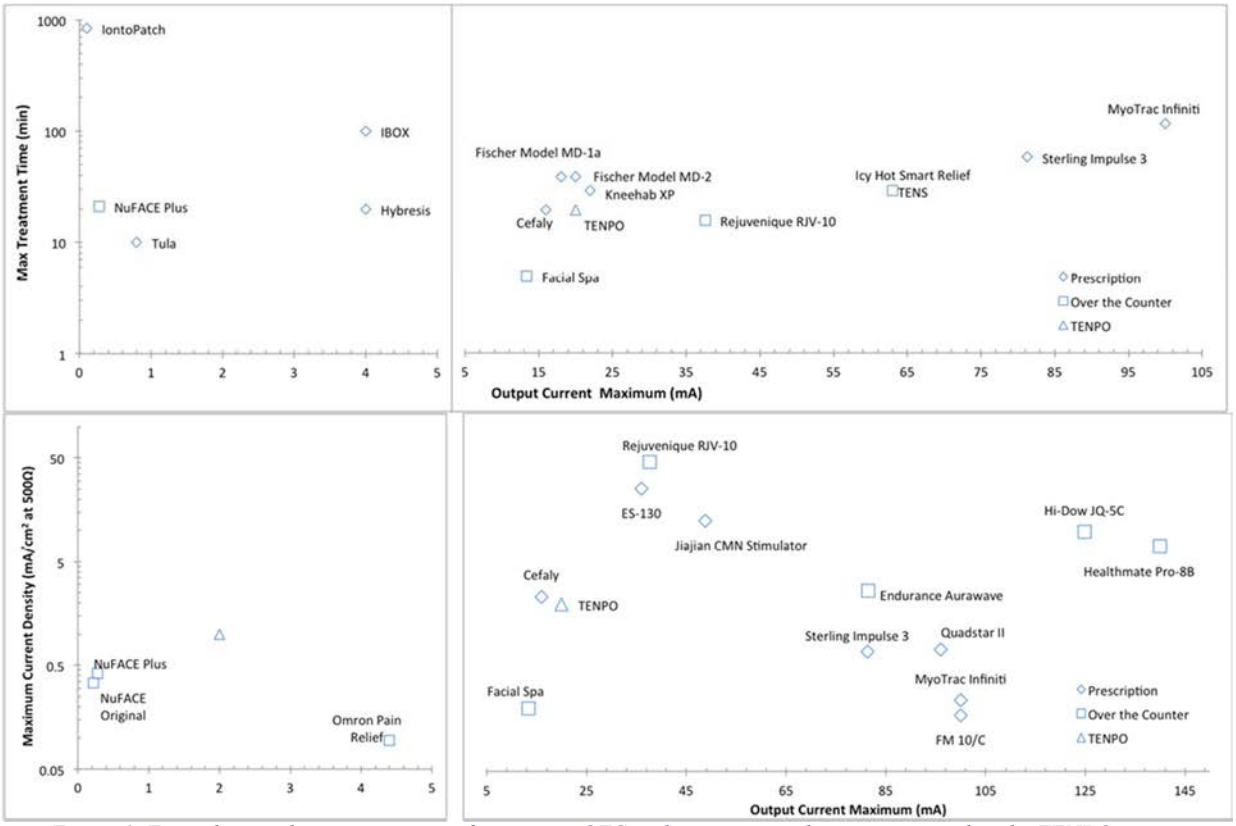

Figure 1. Example stimulation parameters for existing OTC and prescription devices compared to the TENPO targets

In addition to recording chemical brain activity via the FSCV chamber, we may also use it as a route to record electrical activity. This will minimize the number of surgical procedures as well as the amount of hardware on the animal's head.

Throughout Task 1, we will perform two different behavioral tests – an acoustic startle paradigm and a delayed match-to-sample task. The NHPs will be trained on these tasks during the time period when stimulation parameters are being evaluated, and final performance will be assessed after the ideal set of stimulation parameters is identified. This means the NHPs will train/perform these tasks daily (M-F with possible weekends) for no longer than the time period stated in section II. F. of 'Detailed Use of Animals' in this protocol. We also plan to measure pupil diameter and facial skin temperature will be recorded using infrared cameras. Heart rate will be recorded using an optical sensor. This may be measured during all tasks and procedures described in this protocol.

The acoustic startle paradigm is based on previous work in NHPs [redacted]
The test involves the NHP holding its hand down on a pressure pad, while attending to a touchscreen in front of itself. A visual stimulus appears on the screen to signify the start of a trial; the subject must touch the image to initiate the trial. After trial initiation, one of two types of trials are presented. On "startle" trials (a majority of trials), a single acoustic startle stimulus (white noise) will be presented at variable intervals for no longer than 100 ms at no greater than 120 dB. On "prepulse" trials (a minority of trials), an acoustic prepulse stimulus (white noise) will be presented for no longer than 100 ms at no greater than 120 dB, followed by a startle stimulus. Although seemingly quite high, the maximum acoustic intensity for these stimuli is akin to that of an emergency siren. Further-

more, the stimuli are presented at intervals so short in duration we do not anticipate they will induce any significant losses in hearing. For example, OSHA guidelines stipulate that a worker can only be exposed to 115 dB sounds for 15 minutes/day (https://www.osha.gov/SLTC/noisehearingconservation/loud.html). Gunshots, (a prototypical sound requiring hearing protection, are brief, but typically run 170-190 dB). 100 ms tone bursts up to 120 dB should not prove problematic. Noise levels will be calibrated prior to each daily session. We will begin these trials with a startle stimulus of 95 dB, increasing only if needed to elicit the desired LC response. Experimenters should not require personal hearing protection as the acoustic stimulus will be delivered in a closed room (the NHP can be monitored through a window in the door). If the NHP maintains pressure on the pressure pad throughout the trial, after the startle stimulus, the NHP receives a fluid reward. If the NHP removes its hand from the pressure pad at any time between trial initiation and the startle stimulus, the trial is aborted, and no reward is given. An intertrial interval of no more than 60 s from startle stimulus onset occurs regardless of reward delivery.

The delayed match-to-sample (DMS) visuospatial working memory test presented is based on previous work in NHP models [redacted] Each trial initializes after the NHP holds its hand down on a pressure pad, that then activates a primary visual stimulus unique to that trial appearing on the screen. When the subject touches the screen, the trial moves into the interstimulus delay period. The interstimulus delay will be gradually increased throughout training to a maximum of 60 s. Once proficiency at a longer interstimulus delay has been achieved, then the interstimulus delay will be varied between trials. Following the interstimulus delay, at least two secondary visual stimuli will appear evenly spaced throughout the screen. One or more stimuli, the “non-match”, will be different than the primary visual stimulus, and one, the “match”, will be identical to the primary visual stimulus. Touching the “match” stimulus will constitute a correct response, and the NHP will receive a fluid reward. Touching the “non-match” stimulus will yield no reward. Correct and incorrect responses will be followed by shorter and longer intertrial intervals, respectively, to further encourage positive responses.

Task 2: NHP Somatosensory Stimulation

The aim of this task is to determine the extent to which TENPO can drive neuroplasticity in a somatosensory stimulation task. This effort extends findings in auditory cortex plasticity driven by repetitive auditory stimulation paired with a conditioning input to the vagal nerve [redacted] To the extent that this phenomenon is generalizable both to different cortical areas and to conditioning stimuli on different cranial nerves, the options for providing TENPO to enhance learning and performance and the task domains across which TENPO can be effective are dramatically broadened.

Two NHPs will each receive chronic cortical recording arrays in the somatosensory cortex. Once we have determined that those arrays are successfully recording neural activity in the somatosensory cortex that is responsive to mechanical stimulation of the hand, we will install 8 channel longitudinal intrafascicular electrode (LIFE) arrays in the ulnar, radial and/or median nerves. Initially we will stimulate on each channel of the LIFE arrays and record the evoked responses in the somatosensory arrays. Over the next month, we will quantify the number of cortical channels that respond to Repetitive intrafascicular microstimulation (rIMS) on each LIFE

channel at 10, 30, 60, and 90 Hz, and the tuning function for each responsive cortical channel in terms of stimulus. Once we've identified which channels are responsive, the stimulation regimens, for example, will involve stimulation at 30 or 60 Hz delivered every 30 s for a total of 300 stimuli per day, and we will repeat this regimen 5-7 days/week for about 20 days. Similar regimes will be employed as they are discovered from Task 1.

For combined rIMS/TENPO stimulation, we will use TENPO parameters (electrode location, stimulus waveform) as determined from Task 1. Each peripheral nerve stimulus will be paired with TENPO just prior to and for the duration of each stimulus. In all other respects, the experiments will be identical to rIMS only stimulation.

Task 1 will begin before task 2, but the two will be conducted together. We will continue to adjust TENPO parameters based on what we continue to learn from task 1.

In order to more definitively target the locus coeruleus, we may administer an antihypertensive drug, Clonidine. The dose of Clonidine (20 µg/kg) will be injected intramuscularly by PI staff. Clonidine inhibits noradrenergic cells, which are the target of our recording in the locus coeruleus. Observing a reduction in a recorded cell's activity due to clonidine injection would reinforce that we are in the correct location and recording from the correct cells. Injections will be separated by at least 48 hours, and veterinary staff will always be made aware prior to administration. Clonidine is generally considered safe. In humans, common side effects include dizziness, drowsiness, dry mouth, and constipation [redacted]

We may elect to dose the primate with a eugeroic drug, Modafinil, to induce wakefulness/alert behavior and observe a change in LC response, but not significantly affect sleep. Literature recommends an oral dose to be approximately 10 mg/kg, which is our initial target within a 6-32 mg/kg range [redacted] Modafinil has been safely administered to rhesus macaques with minimal nocturnal activity at up to 64 mg/kg when administered at 1700 hours once per week [redacted] Most of the nocturnal activity was seen in the first third of the night, which can be avoided with earlier dosing. For our experiment, Modafinil would be administered on days we plan for those neural recordings, with at least one day of no administration prior to recording. Maximum dosing schedule would be Monday-Wednesday-Friday, with neural recordings obtained within 4 hours post administration of Modafinil. Administration would not occur after 1400 hours to avoid sleep disruption. If significant sleep issues are observed with the primate via video recordings, dosage concentration would be reduced first to a minimum 6 mg/kg, and dosage frequency reduced second, adding additional days of no administration between experiments until no significant disruptions of sleep are observed. Cameras mounted within the animal holding rooms will be used to record time lapse video to observe the animal on nights after administrations. Considering primate safety, there have been no reported lethal overdoses of Modafinil, and the canine LD50 is ~400 mg/kg [redacted] A human narcolepsy trial of 200 mg or 400 mg per day for 9 weeks produced no withdrawal effects. This was followed by open label studies with continued tolerable effective use up to 40 weeks and no dependence formed [redacted]

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

Estimated duration (in months)	Action
2	Quarantine
2	Pole/collar training plus pedestal implant
1	Chair training
12 to 24 (per study)	Behavioral training/recording

The timelines above reflect the estimated duration of events; actual duration may vary.

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

CITATIONS:

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?
   This study is to develop methods to stimulate the release of neuromodulators from certain areas of the brain in order to optimize human performance. At the present time, we do not have any methods outside of live vertebrates to test what we described in Section II Part B. This study is also a direct pre-cursor to human testing. We are using live vertebrates to test and develop stimulation parameters so we may test this technique in humans.

2. Why are you using the requested species rather than other species?
   We have chosen this primate model because there has been substantial background work in understanding the neural structures, particularly underlying somatosensory sensation, but other brain structures of interest as well, and those are thought to be quite similar to human analogs. In addition, there is a substantial literature on the locus coeruleus in the macaque [redacted]

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.
   The first two animals will be used partly to develop techniques and to gain some insight into how we expect the system to respond to TENPO stimulation of the cranial nerves, allowing us to develop a set stimulation schedule for the next two animals in a somatosensory stimulation paradigm . The data arising from these animals we expect will be enough for publication. In the second two animals, we will measure cortical activity and the activity from the peripheral nerves of the arm in response to stimulation. Here, we focus more on the quantity of cellular activity, rather than animal numbers. We plan to start with the first 2 animals, using data collected to refine the chronic experiment, but we plan to continue to collect data from the acute animals after we begin the chronic experiment phase. We will be conducting both experiments simultaneously, therefore, we will need to work with a total of 4 animals.

Experiment	Number of animals
Acute experiment	2
Chronic experiment	2

   Two monkeys is the academic norm for this type of research. To see papers using only two animals, please see [redacted]

4. What refinements, if any, have been made to reduce the number of animals used and the potential detrimental effects on the study animals?
   The first two animals are being used specifically to learn how to minimize detrimental effects on our chronic subjects. 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. All animals will be acclimated to any restraint devices needed for these experiments.

III. EMERGENCY CONTACT

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

Name:
Office Phone #
Home Phone #
Cell Phone #:

[redacted]

IV. DUPLICATION AND ALTERNATIVES PLEASE READ ALL INSTRUCTIONS.

The Animal Welfare Act requires that you document your justifications with data from **two** or more sources. **One source** must **be a set of searches of a relevant database: name the database searched, the keyword and keyword combinations searched, the date the search was performed and the date range searched. The second source can be a set of searches of a second relevant database, or consultation with a laboratory animal science veterinarian, or courses/meetings/consultations with qualified personnel.** Sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge of the availability of alternatives in the specific field of study. Examples of appropriate databases to search include PUBMED, Web of Science, or Animal Welfare Information Center (AWIC – recommended for USDA-covered species; http://awic.nal.usda.gov/literature-searching-and-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*): 8.22.2019
Database(s) used: PubMed
Publication years covered by the search: 1975 - 2019
Keyword combinations used: transdermal neuromodulation, reticular activating system and behavior, locus coeruleus and norepinephrine, nucleus accumbens and dopamine

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*): 8.22.2019
Database(s) used: PubMed
Publication years covered by the search: 1975 - 2019
Keyword combinations used: primate neurophysiology alternative, primate restraint alternative, non-animal model, electrode array implantation alternative, peripheral nerve implant alternative

Keywords searched in various combinations of 1 to 3 words

C. **Results of literature search for alternatives**: Comment on the application(s) of any identified alternatives (found with your search terms, including how these alternatives may be or may not be incorporated to modify a procedure to either lessen or eliminate potential pain and distress.

All protocols must complete this section and must describe how the literature search results relate to painful procedures and alternatives to animal use. You must include sufficient information for the IACUC to determine that a reasonable, good faith effort was made to determine the availability of alternatives. If the search identified any alternative methods (ones that could be used to accomplish the goals of the animal use proposal), you must clearly explain and justify why this alternative cannot be used.

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

Various combinations of these keywords were searched, none of which provided any viable alternatives to animal use or the restraint/training methods as discussed below. In addition, no publications were found describing duplicative work related to our proposed research.

Because of the requirements enumerated in sections II.C.1 and II.C.2, we have not identified replacements for the animals used. We continue to look for alternatives to refine our procedures which allows us to reduce the number of animals required to achieve publishable results; however the core questions we are addressing cannot at this stage be effectively addressed using alternative methods. We have yet to identify a means to get adequate neural signals using methods other than the cranial implant of electrode arrays.
In regard to restraint, as many articles discuss, training can be used in place of restraint or to relieve anxiety during restraint. We use positive reinforcement training for many of our restraint practices. See the IACUC SIGs "Pole and Collar Shaping Plan" and "Chairing Shaping Plan" for information regarding chair restraint. The head restraint is necessary in our tasks as the monkey's head must be perfectly still for recordings. However, they are trained to accept the restraint and are willing to do so. The same can be said for the arm restraint, this is only used in the tasks that require it. The monkeys are willing and cooperate in all of our restraining practices. Food rewards are given during and after each restraint session.

D. Describe any other procedures (e.g., participation in meetings, review of journals) that are used to explore and evaluate alternatives: We are routinely active in searching the literature, attending meetings, and speaking with our collaborators and colleagues about new means to accomplish our research aims.

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			4		4

*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] 8.28.2019

Principal Investigator –Print Date

[redacted] 8.28.2019

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 (http://researchintegrity.asu.edu/training/useofanimals) 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		Species with which individual will have direct contact ("none, "all", or list species)	FOR IACUC USE ONLY Training Confirmation
			What procedures will each person be doing on live animals **under supervision only**?	For which procedures is each person **Level 3 certified** at the time of protocol submission?		
[redacted]	PI	[redacted]		Perform surgeries, recording, poling, restraining, training when needed.	*Macaca mulatta*	Basic 5/2016 Macaque 6/2017 OHSP
	Post Doctoral Researcher			Perform surgeries, recording, poling, restraining, training when needed.	*Macaca mulatta*	11/2018 OHSP
	Lab Coordinator			Poling, restraining, assist/perform surgeries, maintain cleanliness of implants, training	*Macaca mulatta*	11/2018 OHSP
	Associate Professor		Assist with surgeries		*Macaca mulatta*	Basic 11/2016 Macaque 11/2018 OHSP
	Graduate assistant		Assist with surgeries	Poling, handling, maintain cleanliness of implants	*Macaca mulatta*	2/2019 OHSP
	Research scientist		Assist with surgeries	Poling, handling, maintain cleanliness of implants	*Macaca mulatta*	3/2019 OHSP

[redacted]	Associate Professor/Surgeon		Perform nerve implant surgery. Anesthetized animals only.		*Macaca mulatta*	Visiting surgeon – training not required; completes OHSP only when needed
	Neuroscientist		Assist with recordings/data collection. Anesthetized animals only.		*Macaca mulatta*	Visiting surgeon – training not required; completes OHSP only when needed
	PhD Student		Assist with data collection, animal handling, poling, training, implant maintenance		*Macaca mulatta*	5/2019 OHSP
	Student Assistant	[redacted]	Assist with data collection, animal handling, poling, training, implant maintenance		*Macaca mulatta*	5/2019 OHSP
	Student Assistant		Assist with data collection, animal handling, poling, training, implant maintenance		*Macaca mulatta*	5/2019 OHSP
	Student Assistant		Assist with data collection, animal handling, poling, training, implant maintenance		*Macaca mulatta*	5/2019 OHSP
	Student Assistant		Assist with data collection, animal handling, poling, training, implant maintenance		*Macaca mulatta*	5/2019 OHSP
	Student Worker		Handling, training, implant maintenance		*Macaca mulatta*	6/2019 OHSP
	Student		Assist with recordings/data collection		*Macaca mulatta*	7/2018 OHSP
	Student		Assist with recordings/data collection		*Macaca mulatta*	8/2018 OHSP
	Student		Assist with recordings/data collection		*Macaca mulatta*	7/2018 OHSP

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

[redacted] has more than 25 years of experience with primates including handling, training and performing surgeries.

[redacted] has more than 20 years of experience with primates including handling, training and performing surgeries.

[redacted] has more than 8 years of experience handling, training, and assisting/performing surgeries with this species.

[redacted] has been working with primates for over 14 years, and has 5 years of experience handling, training and assisting with surgical procedures involving this species.

[redacted] has about 5 years of experience handling and training this species.

[redacted] has more than 6 years of experience handling and training non-human primates. He previously worked in the lab and has recently returned with a new role on another protocol. We are requesting his addition to this protocol so he is available to assist with surgeries and NHP care as needed.

[redacted] specializes in hand surgery, peripheral nerve reconstruction and microvascular surgery at [redacted] He will only work with anesthetized animals.

[redacted] has been carrying out neurophysiological experiments, largely in rats, for 30 years. He will only work on anesthetized animals.

[redacted] are relatively new to working with this species. All are currently training with [redacted] and/or [redacted] (Level III certified handlers). They will not be handling any animals on their own until they have completed training and Level III certifications have been submitted.

[redacted] has approximately 1 year of handling and surgical experience [redacted] has 2 years of handling and surgical experience [redacted] also have neurochemical recording experience with non-human primates [redacted] has not worked with NHP's and will be trained in necessary handling procedures by either [redacted] or Dr [redacted] (both Level III certified handlers). All three are visiting from another institution and will perform all work under the supervision of either [redacted] or Dr. [redacted] These individuals will be working with our lab to implement a new electrode that they produce.

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: 4

C. Sex: M Age or Weight Range: 3-20kg

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 antibody production?
- ☒ No. Proceed to section II. B.
- ☐ Yes. Complete the following table.

Injection:

Volume of injectate	Adjuvant	Route	Min. Frequency	Max. # of injections

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 only be available at limited times during the day, first during behavioral sessions and then at the end of the day after the animal has worked. On days when animals are not working, their water allotment is split between the AM and PM. Amounts of water provided will vary based on the animal's weight, current work regimen, and habits. This water restriction paradigm is used to provide an incentive for work. Details can be found in the IACUC-approved 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 established baseline will be reported to the veterinarian and the animal will be provided additional water, moistened biscuits, or produce/forage with a higher fat content. The course of action will be determined on an individual basis, and in consult 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 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:

MRIs provide non-invasive imaging to help design and determine proper placement of implants, as well as possible confirmation of implant placement and integrity. 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.

The acoustic startle paradigm will produce very short (100 ms) bursts of up to 120 dB (beginning at 95 dB and increasing incrementally until we see an LC response). This beginning dB range is based on previous startle reflex research in humans [redacted] OSHA allowance for 120dB would be 7.5 minutes per day, which we will never approach (this would equate to ~4500 trials in a single day). For more information on this task, please see Section II. B.

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 a primate chair during testing for a maximum of 6 hours, up to 7 days a week, but usually 5. This duration of time is necessary in order to drive electrodes to the target location very slowly. This ensures the safey of the animal and the longevity of the equipment. Breaks and food treats will be given periodically. 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". During recording sessions 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 on 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. In order to prevent the animals from accessing implanted devices, an arm restraint may be used to limit the use of one arm. The arm restraint consists of a metal tube that one arm is placed inside. This restraint will only be used while the monkey is performing a task. The animals are gradually acclimated to each type of restraint prior to extended periods of use.

After the peripheral nerve surgery, the NHP may have a connector on the upper arm. This connector is attached to the humerus and extends through the skin. The addition of a primate jacket [redacted] helps to prevent infection, as well as deterring the primate from picking at the skin around the implant. The jacket also protects the connector from getting caught on the cage and possibly loosening from the bone. When in use, the jacket will be worn by the NHP at all times and will be changed and washed every 2 weeks, or sooner if it becomes soiled or damaged. The animal will be acclimated to the jacket prior to surgery, beginning with short periods of time (15-30 minutes) while the NHP is in the restraint chair. Once the NHP can keep it on calmly and comfortably, it will be kept on overnight and removed the next time the animal is chaired. We will continue this a few times per week until the surgical procedure.

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 using a disinfectant agent such as chlorhexidine. This is completed minimally once every 7 days for implants without an open craniotomy, and twice per week for any open craniotomy chamber. 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 veterinarian.

For monkeys with open craniotomies, we may use a surgical grade silicone elastomer [redacted] 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.

[redacted] comes in a dual-syringe applicator. The components are mixed automatically as they are dispensed from the applicator. The product is shipped with mixer tips that can be gas sterilized prior to use in order to ensure bacteria free application.

After each recording session, the inside of the chamber is flushed with saline, dried thoroughly with sterile cotton tip applicators, and enough [redacted] is deposited to cover the tissue and surrounding bone ledge inside the chamber. At room temperature, [redacted] cures within 2-3 minutes. Following silicone application/curing, the chamber will be hermetically sealed with a lid that has been fitted with a rubber o-ring in order to minimize the possibility of outside contamination. Before the next recording session, the chamber is opened and the old plug is removed with a sterilized elevator. The elevator is used to pull the edges of the elastomer away from the chamber wall and allow the plug to be easily removed without damaging the underlying tissue.

Twice per week, the aforementioned saline flush will be replaced with a full chamber cleaning as described in the SIG for NHP Implant Maintenance.

Any animal that requires an elastomer seal, but is not working consistently on a task will have the chamber managed in the follow manner:

•The chamber will be checked daily for 3 days after the initial elastomer application, to verify the integrity of the seal and ensure no fluid has built up within the chamber.

•If no fluid is seen after 3 days of checking, the chamber will be checked twice weekly during chamber cleaning.

•If fluid is present in the chamber at any time, this indicates an incomplete seal has taken place. In this event, the old elastomer is removed, the chamber is cleaned and dried, and a new seal is applied. Three days of daily checks begin again at this stage.

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

See the IACUC-approved 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 animals will have their identification numbers 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
Baytril	5 mg/kg	PO or IM	Antibiotic	SID-BID based on vet assessment	Y	N
Betadine	N/A	Topical	Clean implant; 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	20-30 mg/kg	IV or IM	Antibiotic	Every 4 hours intraoperatively, BID based on vet	Y	N

				assessment		
Cephalexin	20-30 mg/kg	PO	Antibiotic	BID based on vet assessment	Y	N
Chlorhexidine	N/A	Topical	Clean implant; Disinfect surgical sites	As needed	Y	N
Clonidine	20 µg/kg	IM	Inhibit noradrenergic cell activity to assist in targeting LC	Every 48 hours, during data collection	Y	N
Cyanoacrylic glue (e.g., DuraGen/DuraSeal)	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
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% glycerin	4 L	IV	Perfusion	Once	N	N
Gadolinium	0.1 mmol/kg	IV catheter	3D brain modeling prior to implant placement	Once, prior to MR angiography scan	Y	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	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

Modafinil	6-10 mg/kg	Orally in syringe, mixed with juice or gum	Increase wakefulness	Mon, Wed, Fri, 4 hours prior to recording. No later than 1400 to avoid sleep disruption	Y	Y
Oxymorphone	0.07-0.15 mg/kg	SC, IM or IV	Analgesic	As needed, based on vet assessment	Y	Y
Pentobarbitol- containing euthansia solution	86-120 mg/kg	IV	Euthanasia	Once	Y	Y
Phosphate buffered saline	4 L	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
Puralube	Dab	Topical	Prevent corneal dessication	Once, 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
Silicone elastomer (e.g., [redacted]	N/A	Topical	Seal craniotomy tissue	As needed	Y	N
Tramadol	1-2 mg/kg	PO	Analgesic	SID-BID, as needed based on vet assessment	Y	Y
Triple antibiotic ointment/Silver sulfadiazine	Dab	Topical	Antbiotic	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
Herpes B*					19-872

*Herpes B is not being used in animals, but can be transmitted to personnel if there is an NHP bite/exposure.

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.

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

IV. DETRIMENTAL SEQUELAE

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

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

Dehydration from fluid regulation	1%	*Ad libitum* access to fluids.
Implant Infection	10%	Clean with hydrogen peroxide, betadine or chlorhexidine. Other treatments per veterinary recommendation. See IACUC-approved SIG "NHP Implant Maintenance".
10% body weight loss from fluid regulation	10%	Refer to the SIG "NHP Fluid Regulation" for details on modifications for weight gain.
Loss of appetite due to fluid regulation	50%	Avoid dehydration, high calorie supplements, monitor body weight, moisten biscuits if needed to stimulate appetite, increase water allotment if needed.
Post-operative pain	75%	Analgesia regimen, see IACUC-approved SIG "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 an 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	4-8%	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	4 L	IV	N	Used in coordination with perfusion
10% Formalin ± 20% glycerin	4 L	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 1: ANTEMORTEM SPECIMEN COLLECTION

I. BLOOD COLLECTION

A. Will blood be collected?
- ☒ No. Proceed to section II.
- ☐ Yes. Complete the following.

Site	Volume (ml)	% BW	Max. # of collections	Min. Interval

B. Will anesthetics, sedatives, or other drugs be used during blood collection?
- ☐ No. Proceed to section I. C.
- ☐ Yes. Complete the following.

Drug	Dose	Route	Purpose

C. Describe the methods used to draw the blood including physical restraint, if any.

D. Provide scientific justification for blood collection and justification for the frequency of it.

II. OTHER TISSUE/BODY FLUID COLLECTION

A. Will other tissues or body fluids be collected prior to death?
- ☒ No. Appendix 1 is completed.
- ☐ Yes. Complete the following. Surgical procedures should be described more fully in Appendix 2.

Tissue/Fluid	Site and Method	Amt	# of collections	Min Interval

B. Will anesthetics, sedatives, or other drugs be used during tissue/body fluid collection?
- ☐ No. Proceed to section II. C.
- ☐ Yes. Complete the following.

Drug	Dose	Route	Purpose

C. Describe the methods used to collect the samples, including physical restraint, if any.

D. Provide scientific justification for the sample collection(s) and justification for the frequency of it

APPENDIX 2: SURGICAL PROCEDURES

I. GENERAL INFORMATION

A. Species
Macaca mulatta

B. Surgical Procedure(s)

1. Pedestal implant
2. Post procedure
3. Cuff electrode implant (if necessary)
4. Chamber implant
5. Right Hemisphere electrode array cortical implant
6. Left Hemisphere electrode array cortical implant
7. Bone Plate
8. Left arm peripheral nerve array implant
9. Right arm peripheral nerve array implant
10. Implant removal
11. Vasectomy
12. Repair procedures as needed

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

II. PRE-SURGICAL CARE

A. Will the animals undergo pre-surgical fasting?
☐ No. Proceed to section III.
☒ Yes. Provide the details:
Animals scheduled for surgery are fasted the night before the procedure. The animal's full PM ration is offered early in the afternoon, and any remaining food is removed from the cage before DACT staff leaves for the day.

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 seven surgically implanted devices may be performed on each animal (listed above). Monkeys will either receive the chamber implant or the cortical array implant. The arm peripheral nerve arrays will only be implanted in the animals that receive the cortical array implants. All animals will receive the pedestal/post implants.

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, with the chamber, nerve cuffs, or cortical implants second. The peripheral nerve implants will be performed last. If both hemisphere cortical implants 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 twelve 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 the protocols or any modifications to the surgical procedures as currently described in the protocols 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. Ophthalmological 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 $ETCO_2$, 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 is applied to the ear bars prior to use to provide local pain relief. After the head is shaved and scrubbed with novalsan/alcohol, a sterile field is established with the use of surgical drapes. For all procedures, the subcutaneous tissue and skin (if applicable) will be closed with an absorbable suture such as Monocryl®, PDS®, or Vicryl® 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, an ~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.

3. Cuff electrode implant

We will cut small incisions over one or more branches of the trigeminal nerve as it enters the face. The nerve will be carefully exposed using blunt dissection and cautery of connective tissues. Once the nerve has been identified, we will use a subcutaneous tunneling tool to open a route from the incision back to the location of the chamber that will be implanted. Leads from the cuff electrodes will be routed through the tunnel to the location of the chamber. Once the leads are in place, the cuff electrode will be wrapped around the nerve, and gently secured in place with sutures. Tissue will then be closed in layers over the implanted electrode.

4. Chamber implant

This procedure will 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. The mount is a custom machined round piece of polyether ether ketone with legs for attaching the same screws that are used in attaching the pedestals in Procedure 1. The recording chamber has 4 legs and a profile underneath that is designed to match the contour of the skull. In this procedure, we identify the location of the chamber with the animal in a stereotax, and then remove a piece of skin 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. When the tissue has been sufficiently cleared, we will cut a 15 to 20 mm diameter craniotomy on the inside of the chamber and discard the bone. We will then secure the chamber to the bone using bone screws. We close by pulling the remaining skin up over the legs of the chamber, and if necessary add just a few sutures based on veterinary recommendation to hold the skin in place.

5 and 6. 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] is 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. The [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 Gelfoam 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.

7. Bone Plate

If we implant peripheral nerve arrays that require a wired connector, the connector will extend through the skin to allow us to connect the array to our computer system. To ensure stability and integrity of the connector, we will perform a bone plate procedure at least 6 weeks prior to the peripheral nerve implant. The six-week time period allows for osseointegration of the bone around the metal plate. For the bone plate procedure, a titanium plate is implanted into the humerus and attached using orthopedic titanium screws. Once affixed to the bone, a protective plastic cover is placed over the plate, so that the bone cannot completely grow over the face of the plate. The skin is then sutured over the plate.

8 and 9. Electrode array peripheral nerve implantation

We will perform a dissection of the lower arm, exposing the three distal branches of the brachial nerve: ulnar, medial, and radial nerves. The surgery will be performed by

[redacted] of [redacted] has an established practice in "nerve transfer" surgeries. In these surgeries, the fascicles of nerves are dissected out and "reassigned" to replace the function of damaged nerves. Up to three ~4 inch-long incisions will be needed for the implantation of the electrodes. The implants will be surgically placed under intermittent tourniquet control or electrocautery in order to limit bleeding. The tourniquet will not exceed 30 minutes of continued use in order to avoid anoxic damage to the nerves. Once the arrays have been implanted, we will either attach systems to manage wireless communication between the electrodes and outside control devices, or implant a wired connector to the humerus. The wireless package will likely consist of a small unit that is implanted in the forearm near the electrodes, and may also include a power device implanted more remotely, for example in the chest, and a wire communicating between the two. Once the arrays have been implanted, the skin will be sutured in layers, and the animal allowed to recover.

10. 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.

11. Vasectomy

Every attempt is made to transfer animals to a veterinary approved animal retirement facility after use. In some instances, it is necessary to vasectomize males so that they can be transferred to a retirement facility and housed with females. Vasectomies will be performed by ASU veterinary staff using procedures chosen at the discretion of the ASU veterinarian.

12. 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 remove it. These surgeries will always take place in consultation with the veterinary staff.

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)	3-15 mg/kg	IM	Y
Midazolam (5 mg/ml)	0.05-0.5 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)	2-5 mg/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	Frequency	Purpose	Is this a DEA controlled substance (Y/N)?
Betadine/Chlorhexidine	N/A	Topical	Once, as needed	Disinfect surgical sites	N
Bupivacaine	1-2 mg/kg	SC	Once, as needed	Local anesthetic	N
Cefazolin (330 mg/ml)	20-30 mg/kg	IV	Every 4 hours intraoperatively	Antibiotic	N
Cyanoacrylic glue (e.g., DuraGen/DuraSeal)	As needed	Extradural	Once, as needed	Close/seal dura mater	N
Dexamethasone (2 mg/ml)	0.25-2 mg/kg	IM or IV	As needed	Reduce inflammation	N
Doxapram	2 mg/kg	Topical (tongue) or IV	As needed based on vet assessment	Stimulate breathing	N
Epinephrine	0.2-0.4 mg/kg	SC, IM or IV	As needed based on vet assessment	Stimulate heart, vasoconstriction	N
Gelfoam	Cut to size	Topical	Once, as needed	Hemostasis/ Seal surgical holes	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	Continuous during surgery	Fluid support	N
Lidocaine containing gel/cream	Dab	Topical	Once	Local anesthetic	N
Mannitol (200 mg/ml)	0.25-2.2 g/kg over 20 minutes	IV	As needed	Reduce intracranial edema	N

Puralube	Dab	Topical	Once, as needed	Prevent corneal dessication	N
0.9% NaCl Solution	5-15 ml/kg/hr	IV	Continuous during surgery	Fluid support	N
3% NaCl Solution	250 ml bolus over 30 minutes	IV	As needed	Reduce intracranial edema	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. Sporocidin 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 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/sig) 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	Used PRN based on veterinary assessment	Y
Buprenorphine SR (1 mg/ml)	0.2 mg/kg	SC	Once, based on veterinary assessment	Y
Meloxicam (5 mg/ml injection; 1.5 mg/ml oral)	0.1-0.2 mg/kg	SC or PO	SID/variable duration based on procedure	N
Oxymorphone (1 mg/ml)	0.07-0.15 mg/kg	SC, IM or IV	Once, PRN based on veterinary assessment	Y
			SID-BID/	

Tramadol	1-2 mg/kg	PO	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	Frequency	Purpose	Is this a DEA controlled substance (Y/N)?
Cefazolin (330 mg/ml)	20-30 mg/kg	IM	BID/variable duration based on procedure	Antibiotic	N
Cephalexin (50 mg/ml)	20-30 mg/kg	PO	BID/variable duration based on procedure	Antibiotic	N
Baytril (Enrofloxacin) (22.7 mg pill or 22.7 mg/ml)	5 mg/kg	PO or IM	SID/variable based on procedure	Antibiotic	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 attach posts to the implanted pedestals which allows for head stabilization. This may be performed in conjunction with pedestal implantation. The animals that undergo the chamber implant will not receive the cortical array or peripheral nerve array procedures, and vice versa. The cortical array surgeries involve implantation of

systems that allow us to record and/or stimulate the activity of multiple neurons simultaneously. 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. In the case of a wired peripheral nerve implant, we will first need to implant the connector mounting plate (bone plate) to allow time for it to osseointegrate for stability. The peripheral nerve electrode array implant is conducted at least 6 weeks afterwards. Duration between other 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 an additional surgical procedure to remove the implants and vasectomize the animal if it is a male.

IACUC Protocol Trackable Components Checklist

Protocol #: 20-1743R If for amendment, amendment #:

PI: [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 9/20/19

☐ 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 metal tube that one arm is placed inside. Arm restraint will only be used while the monkey is performing a task.

☒ 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:

Surgerie(s): A maximum of 7 surgically implanted devices per animal. Procedures include:

1. Pedestal implant
2. Post procedure
3. Cuff electrode implant (if necessary)
4. Chamber implant
5. Right Hemisphere electrode array cortical implant
6. Left Hemisphere electrode array cortical implant
7. Bone Plate

IACUC Protocol Trackable Components Checklist

8. Left arm peripheral nerve array implant
9. Right arm peripheral nerve array implant
10. Implant removal
11. Vasectomy
12. Repair procedures as needed

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), Acoustic startle paradigm (100 ms bursts of up to 120 dB sound), CT scan and radiographs (X-ray radiation)

☐ Non-Centralized Animal Housing

Location:

Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

SURGERY PREP
To Dos

DATE: _____

PROCEDURE: _____

2 Weeks Prior:

- ☐ Email all participating individuals to coordinate schedules and choose a date
  - ☐ Send calendar invite once date is selected (Dr [redacted] or [redacted]
- ☐ Look over Surgery Tools checklist
  - ☐ Additional items needed not on list? Y/N
    1. _____
    2. _____
    3. _____
    4. _____
  - ☐ Does anything need to be ordered? Y/N
    1. _____
    2. _____
    3. _____
    4. _____

At least 3 days prior:

- ☐ Make sure any items that need to be sterilized have been packaged and given to [redacted]

The day before:

- ☐ Email DACT/Vet Team to fast the animal the night before the procedure (Dr. [redacted] or [redacted]
- ☐ Gather all supplies needed from the checklist – double check nothing has been forgotten!
- ☐ Ensure that any major adjustments have been made to the stereotax if it is being used, and that all necessary tools are available for changes on surgery day. Minor modifications can be made on the day of.