Amendment 1

Personnel Information 2

Species 13

Are You Using? 14

Funding Sources 16

Rationale 17

Procedures 20

Alternative Search 91

Procedure Relationships 93

Husbandry 94

Animal Disposition 97

Attachments 100

Certifications 101

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Amendment * * *

Amendment

List of Sections (and questions) that have been changed/modified

If you would like to make changes to the information in the protocol, add/remove/update personnel, add/modify a procedure, etc., click on the appropriate section/link on the left side menu.

1. Please provide a brief summary of the proposed amendment:

   Addition of new personnel.

2. Please provide a rationale for the proposed amendment:

   [redacted] **is a rotating graduate student from the Bioengineering program.**

3. Check all that apply. The proposed amendment involves:

   - A change from non-survival to survival surgery
   - A change that results in greater pain, distress, or degree of invasiveness
   - A change in housing and/or use of animals in a location that is not part of the animal program overseen by the ACUC
   - The addition of a new species
   - A change in the objectives of a study
   - A change in the Principal Investigator (PI)
   - A change that impacts personnel safety
   - A change in compounds or dosage of experimental substances which are fundamentally similar to compounds already approved in the protocol, and are documented in the literature regarding safety and toxicity in the same species
   - A change in compounds or dosage of anesthesia, analgesia, or sedation that are consistent with UCB ACUC guidelines
   - A change in euthanasia method to any method approved in the AVMA guidelines (including UCB ACUC euthanasia guidelines)
   - A greater than () 10% increase in the originally approved number of animals of any one species
   - X Changes in personnel (other than PI)
   - Changes in funding sources

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Personnel Information * * *

Principal Investigator

(Must have PI status or Exceptional PI status at UC Berkeley)

Name [redacted]

Title [redacted]

Email [redacted]

Office Phone [redacted]

Lab Phone [redacted]

Emergency Phone [redacted]

Department [redacted]

Mail Code [redacted]

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If "Yes" complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, surgery, monitoring, post-procedural care.

Describe the experience/training this person has had with this/these species and procedures.

Experienced with using procedures since 1999

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, "Investigators, Staff and Students - Basic Course" and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Laboratory Contact

Name [redacted]

Title

Email

Office Phone

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

[redacted]

Lab Phone

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X **Yes** **No**

If "Yes" complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, surgery, monitoring, post-procedural care.

Describe the experience/training this person has had with this/these species and procedures.

Experienced with all procedures since 2014

06/03/2014: CITI Training completed
06/03/2014: Working with Non-human primates in Research Setting
06/03/2014: Streaming videotape - working safely with non-human primates
06/10/2014: Mandatory OHC
06/19/2014: Completed Herpes B Safety Training with [redacted]
06/05/2014: EH&S [redacted] Safety Training
11/25/2014: Aseptic surgery

[redacted] will be certified by OLAC prior to performing surgery independently.

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Other Personnel

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Name	Department
[redacted]	[redacted]
[redacted]	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Other Personnel

Name
[redacted]

Title
[redacted]

Email
[redacted]

Office Phone
[redacted]

Lab Phone

Emergency Phone

Department
[redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: MRI scanning

Describe the experience/training this person has had with this/these species and procedures.

MRI scanning since 2005

8/21/2014: OHC Health information and Herpes B training with [redacted]

3/23/2015: CITI certification "Working with the IACUC"

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Name [redacted]	**Title**
Email [redacted]	**Office Phone**
Lab Phone	**Emergency Phone**
Department	**Mail Code**
Campus Mailing Address	
Will this individual be working directly with animals on this protocol?	X Yes No
If Yes complete the following:	
What species will this person use?:	macaques
Briefly list what procedures this person will perform (a full description of procedures is asked for later).:	anesthesia and peri-procedure care

Describe the experience/training this person has had with this/these species and procedures.

OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

UCB
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Name [redacted]

Title

Email [redacted]

Office Phone

Lab Phone

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing.

Describe the experience/training this person has had with this/these species and procedures.

Trained in procedures since 2017

12/6/16: Mandatory OHC
12/19/2016: CITI Training "Working with the IACUC"
1/31/2017: Herpes B training with [redacted]
1/27/17: EH&S/[redacted] Safety Training

[redacted] will be certified by OLAC prior to performing surgery independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title

Email

Office Phone

UCB
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Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

[redacted]

Lab Phone

Emergency Phone
[redacted]

Department
[redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, surgery, monitoring, post-procedural care.

Describe the experience/training this person has had with this/these species and procedures.

Experienced in procedures since 2018

CITI training “working with the IACUC”: 08/17/17
Occupational Health:
Primate health screening (TB/measles titer): 11/15/17
Measles follow-up vaccine #1: 12/8/17
Measles follow-up vaccine #2: 1/5/17
Risk assessment form: 12/26/17
Herpes B:
EHS202 online bloodborne pathogens training: 12/11/17
Herpes B in-person training: 1/8/17
OLAC training:
EHS201 in-person biosafety training: 8/15/17
OLAC Basic Safety Training: 12/11/17

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name
[redacted]

Title

Email

Office Phone

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

[redacted]
Lab Phone

[redacted]
Emergency Phone

Department
[redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus macaque

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Providing water to water restricted animals

Describe the experience/training this person has had with this/these species and procedures.

Experienced with procedures since 2018

8/1/18: CITI Training completed
8/8/18: [redacted] basic safety training completed
8/14/18: Mandatory OHC medical clearance completed
8/6/18: CITI Working with Non-Human Primates in Research training completed
8/21/18: Herpes B training with [redacted] completed
[redacted] will be certified by OLAC prior to performing anesthesia, surgery or euthanasia independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name
[redacted]

Title

Email
[redacted]

Office Phone

Lab Phone
[redacted]

Emergency Phone
[redacted]

Department

Mail Code

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

[redacted]

Campus Mailing Address
[redacted]

Will this individual be working directly with animals on this protocol?	X Yes No
If Yes complete the following:	
What species will this person use?:	Rhesus macaque
Briefly list what procedures this person will perform (a full description of procedures is asked for later).:	Pole and collar training, handling, behavioral testing, monitoring, post-procedural care. Chronic and semichronic neural recordings.

Describe the experience/training this person has had with this/these species and procedures.

Experienced with procedures since 2016

[redacted] completed Mandatory OHC medical clearance on 2/23/2016.
2/12/2016: CITI Training completed
2/25/2016: Herpes B training with [redacted]
3/8/2016: [redacted] basic safety training.
[redacted] will be certified by OLAC prior to performing anesthesia, surgery or euthanasia independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name
[redacted]

Title

Email
[redacted]

Office Phone

Lab Phone
[redacted]

Emergency Phone
[redacted]

Department
[redacted]

Mail Code

Campus Mailing Address
[redacted]

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Will this individual be working directly with animals on this protocol?	X **Yes** **No**
If Yes complete the following:	
What species will this person use?:	Rhesus macaque
Briefly list what procedures this person will perform (a full description of procedures is asked for later).:	Pole and collar training, handling, behavioral testing, monitoring, post-procedural care. Chronic and semichronic neural recordings.

Describe the experience/training this person has had with this/these species and procedures.

Experienced with procedures since 2015

[redacted] completed [redacted] safety training and Mandatory OHC
8/7/2015: CITI Training completed
8/26/2015: Herpes B training by [redacted]

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title

Email [redacted]

Office Phone

Lab Phone [redacted]

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address [redacted]

Will this individual be working directly with animals on this protocol?	X **Yes** **No**
If Yes complete the following:	
What species will this person use?:	Rhesus macaque

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, monitoring, post-procedural care. Chronic and semichronic neural recordings.

Describe the experience/training this person has had with this/these species and procedures.

Experienced with procedures since 2018.
[redacted] completed Mandatory OHC medical clearance on 8/7/2017.
7/15/2017: CITI Training completed
8/16/2017: [redacted] basic safety training completed.
8/21/2017: [redacted] facility orientation completed.
8/25/2017: Herpes B training with [redacted]
[redacted] will be certified by OLAC prior to performing anesthesia, surgery or euthanasia independently

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title

Email [redacted]

Office Phone [redacted]

Lab Phone

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus monkey

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Behavioral training, maintenance of neurophysiological implants

Describe the experience/training this person has had with this/these species and procedures.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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0 years experience. Student will be trained by PI and current personnel in the lab.

CITI training completed on 8/30/19
Occupational Health completed on 9/10/19
Herpes B training completed on 9/16/19
EH&S training completed on 9/13/19

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Species * * *

Species to be Used

Common Name	Genus & Species	Source
Monkey, Rhesus	Macaca mulatta	OLAC Approved Vendors,Other includes animals that are already part of our existing colony

Species to be Used

Common Name — **Monkey, Rhesus**

Genus & Species — Macaca mulatta

Strain(s) or Breed(s)

Animal Sex — **Male**

Source — OLAC Approved Vendors,
Other includes animals that are already part of our existing colony

Proposed Housing Location — [redacted]

Building Name — [redacted]

Room Number

Maximum number of animals for three year project period — 30

Note: If breeding animals, the maximum number should include breeders plus all offspring produced.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Are You Using? * * *

NOTE: Select either "yes" or "no" for each question. If you select "yes", click on the "Add" button to provide required information.

Are You Using?

NOTE: The questions below are used to identify special circumstances where:

1) Animals are used in teaching

2) Additional oversight by regulatory agencies may be required

3) Coordination with campus compliance committees may be required

4) Personnel health and safety issues need to be addressed

1. Are you using live vertebrate animals for teaching?* N

2. Collaboration with Other Institution(s)* N

Animal transfers or changes in animal ownership between UC Berkeley PIs and collaborators at other institutions must comply with the ACUC policy on Changes in Animal Ownership and ACUC Guidelines on Animal Transportation.

3. Hazardous Agent(s) in Laboratory Animals

a) Infectious Agent(s) * N

Use of BSL-2 or 3 infectious agents in animals (including viral vectors; human cells, tissues or bodily fluids; and infectious select agents) requires approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site .

b) Recombinant DNA * N

The introduction of recombinant DNA/RNA into animals and the generation of transgenic animals require approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site .

NOTE: If breeding animals, create a "Breeding/Genotyping" Procedure and provide additional information and justification (including specific strains and phenotypes).

c) Human Embryonic Stem Cells * N

NOTE: Use of Human Embryonic Stem Cells in animals requires approval by the UC Berkeley Stem Cell Research Oversight Committee (SCRO) and Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the SCRO web site and the EH&S Biosafety Program web site.

1. Do you have SCRO approval? *
2. BUA # *
3. Used In Which Species?

d) Biological Material/Animal Product(s) Not Described Above* N

NOTE: The use of biological materials in rodents must comply with the ACUC Policy on Testing Biologicals used in Laboratory Rodents. The use of human cells, tissues or bodily fluids requires approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site.

e) Toxic Agent(s) * N

This includes the use of carcinogens, reproductive hazards, and other biological toxins (including select agents) in laboratory animals. Standard Operating Procedures (SOPs) must be in place. For guidance, please refer to the EH&S SOP web site.

f) Controlled Substance(s) * Y

NOTE: The Principal Investigator and any individuals using controlled substances in animals must be registered with EH&S prior using these agents. For guidance, please refer to the EH&S Controlled Substance Program web site.

Controlled Substance

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Species	Agent
Monkey, Rhesus (OLAC Vivarium)	Buprenorphine (Buprenex)
Monkey, Rhesus (OLAC Vivarium)	Tramadol
Monkey, Rhesus (OLAC Vivarium)	Ketamine

g) Radiological Agent(s) * N

NOTE: Use of radiological agents in animals, radiation producing devices or lasers requires an approved Radiation Use Authorization(RUA) or Laser Use Registration (LUR) be in place prior to ACUC approval. For further guidance, please refer to the EH&S Radiation Safety Programs web site or Laser Safety Program web site.

4. Non-pharmaceutical Grade Compounds * Y

NOTE: Federal regulations require the use of pharmaceutical grade compounds in animals used for research and teaching unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. Please refer to the ACUC Policy on Use of Non-Pharmaceutical Grade Compounds

Non-pharmaceutical Grade Compounds

Species	Specify Material	Please provide justification for use of non-pharmaceutical compounds
Monkey, Rhesus (OLAC Vivarium)	Muscimol	Pharmaceutical grade are not available.
Monkey, Rhesus (OLAC Vivarium)	SCH23390	Pharmaceutical grade is not available

5. Field Study or Wildlife Study* N

NOTE: Additional procedure-based information for field studies is requested under the Protocol Information section of the Protocol.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Funding Sources * * *

Funding Checklist

If the research is not funded, check the "Not Funded" box below.
If the research is funded, add the funding source to the appropriate table below.

NOTE: Only the Principal Investigator (PI) of the grant or subcontract can add his or her own SPO Funding information in this section. The PI of the grant or subcontract must also be listed in the Personnel Information section of the protocol in one of the following roles: Principal Investigator or Faculty Sponsor, Student or Postdoctoral Investigator, Co-Principal Investigator, Administrative Contact, or Other Contact. Training Grants can be added by anyone in one of the aforementioned roles. For step-by-step instructions, see eProtocol IACUC Quick Guides

Not Funded

SPO - Funding

SPO ID	Sponsor	Sponsor Award ID	Project Title
036460-002	NIH National Institute of Mental Health	1R01MH097990-01A1	Functional architecture of Oribitofrontal Cortex
20181319	NIH National Institutes of Health - Miscellaneous		Frontostriatal Rhythms Underlying Reinforcement Learning
20192354	NIH National Institutes of Health - Miscellaneous		Hippocampal-Orbitofrontal Interactions and Reward Learning

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Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Rationale * * *

Rationale

As you answer the questions in this section, please use language that can be understood by a layperson. r Avoid overly technical terms and define abbreviations.

1. STUDY OBJECTIVES

a) What is the overall aim and purpose of this research or teaching demonstration/exercise?*

This research is directed towards understanding the neuronal mechanisms that permit reward information to control behavior, in particular how does such information integrate with cognitive processes such as maintaining information in working memory, attention, planning, and the formulation of behavior-guiding rules and strategies?

Because the relationship between cognitive processes and neural signals remains unclear, our research measures brain activity at a variety of different scales (including single units, local field potentials and electrocorticography) and targets a wide variety of brain areas.

b) How will the information gained be important to human or animal health, the advancement of knowledge, or the good of society?*

Our research studies the prefrontal cortex, an area that reaches its greatest complexity in primates. Dysfunction of this area has been implicated in many neuropsychiatric disorders, including attention-deficit hyperactivity disorder, post-traumatic stress disorder, obsessive compulsive disorder, alcoholism, drug addiction, pathological gambling, obesity and eating disorders. The aim of our research is to understand the mechanisms and computations performed by prefrontal neurons. By so doing, our goal is to understand how dysfunction of prefrontal cortex causes neuropsychiatric disorders so that we can use this knowledge to design the next generation of treatments.

2. RATIONALE FOR USE OF ANIMALS

a) Why do you need to use animals? Discuss why non-vertebrate alternatives (e.g., tissue culture, invertebrate animal models, computer simulations) are inappropriate or implausible to answer your scientific questions or meet your educational goals.*

The experiments described in this protocol involve standard neurophysiological techniques to record the activity of single neurons in the frontal cortex of awake, behaving animals. Neurophysiology is currently the only way to understand how information is processed within a brain region. Such research is vital to lay the groundwork for future studies that aim to manipulate such neurons pharmacologically, with the aim of providing a therapeutic basis for disorders in which dysfunction of the frontal cortex is implicated.

Such research is unethical in healthy humans since it necessarily involves a very small amount of damage to the neural tissue. Such damage is insufficient to cause cognitive or motor impairments in the animals but, nevertheless, any amount of neural damage would be unethical in humans solely for research purposes. A couple of studies have recorded from the prefrontal cortex of epileptic patients (the cortex was to be excised to control the patient's epilepsy and as such damage was not an issue). However, the interpretation of such studies is complicated since the tissue that is being recorded is abnormal (epileptogenic), or interacts with the abnormal tissue.

Non-invasive methods for studying brain function do not tell us how information is processed within a brain region. For example, neuroimaging techniques such as fMRI and PET can identify whether a region is active during the performance of a task, but do not reveal the nature of that processing. Furthermore, the lack of experimental data regarding the use of reward information in higher cognitive processing precludes the use of computational methods to elucidate the neuronal mechanisms in prefrontal cortex.

b) Why have you selected these particular species (and not others)?*

Experiments will be carried out on adult and juvenile macaques, including cynomologus (Macaca fascicularis), pigtail (M. nemestrina), and/or rhesus (M. mulatta) macaques of either sex, weighing at least 3 kg. Our experiments are designed to examine the effect of reward on cognitive processes such as planning, working memory and decision-making. These experiments have important implications for the problem of addiction, in which rewarding substances such as drugs conflict with long-term plans such as quitting the drug.

Our experiments are focused on the higher cognitive processes instantiated by the prefrontal cortex. This area of the brain is one that has undergone a dramatic increase in size and complexity over the course of mammalian evolution, reaching its greatest complexity in primates, and in particular humans. Indeed, it is virtually non-existent, or at least much more rudimentary, in other mammals, necessitating the use of primates in our research. The choice of the particular species of primate depends on availability. Any of the above three species could be used, as there are no known major differences in the anatomy of the prefrontal cortex between different macaque species. In general, however, M. mulatta would be preferred as they are somewhat more cooperative and easier to train and handle.

3. JUSTIFICATION OF ANIMAL NUMBERS

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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For complete instructions and guidance on how to complete the section on justification of animal numbers, please refer to the ACUC guideline on Justification for Animal Numbers found on the ACUC website.

a) How did you determine that the numbers provided in the Species section of this protocol are the smallest number of animals needed to fulfill the study goals over a three-year period? Please use the table below to graphically describe for reviewers how you arrived at your animal numbers. Regardless of species, please briefly describe the Experiments included in your protocol and complete the table below, FOR THE THREE-YEAR PERIOD OF THE PROTOCOL. Note: Experiments may consist of multiple procedures. For breeding colonies, enter these as a line item, with the total consisting of breeding stock plus offspring NOT used in any studies.

Animal Groups for Procedures

Experiment	Maximum number of groups (Control)	Maximum number of groups (Experimental)	Maximum number of animals per group	Maximum number of replications needed	Total number of animals needed
Electrophysiology and behavior in macaques	0	1	30	1	30

b) Please justify the proposed number of animals being used:

For the macaque experiments, our modern electrophysiological techniques allow hundreds of neurons to be simultaneously recorded in each animal. These methods yield an extremely high sample size of neurons, while minimizing the number of subjects required. Therefore, we will be able to collect far more information per subject than is normally possible in these types of studies. In addition, since we propose to carry out multi-level recordings in these animals, multiple neuronal structures (cortical and subcortical) can be simultaneously investigated in each subject.

We typically have three to five behavioral tasks being used in the lab at any given time. Each task requires at least 2 animals per the standards of the field. Since not all animals are capable of learning all tasks we typically have 3 animals allocated to learning each task, thereby requiring a maximum of 15 animals at any one time. Animals are frequently retrained to learn a new behavioral task with subsequent neurophsyiological recording. This is because it is frequently scientifically useful to compare neural responses across tasks within the same animal. This enables us to control for many of the confounds that might arise by trying to compare across animals (e.g. individual differences physiology and/or behavioral performance).

Since our experiments typically take 2-3 years to complete, the maximum number of animals that might be required in a three year period is 30. In other words, the three year period would begin with one set of five experiments involving 15 animals carried over from the previous approval period, and these animals would be gradually replaced during the three year period with a second set of 15 animals, as one study ended and another study began. Thus, a maximum of 30 animals would be used in a three year period.

c) Method Used to Determine Group Size (check all that apply):

X **Statistical estimates; please describe the power analysis and all other statical analyses used:**

A statistical power analysis is not appropriate for macaque studies. Two macaques per experiment are the minimum necessary according to the standards of the field, and to provide some validation of each individual's results. A third macaque may be needed to increase the number of recorded cells in the event of poor cell yield in the recordings. There are typically five concurrent studies, with each study lasting 2-3 years. Thus, a maximum of 30 animals would be required over a three year period.

This is a pilot study, as similarly established studies do not exist. The proposed study will use a small number of animals to determine the feasibility of a larger study.

Studies cited in the literature; please provide the literature citations here or as an attachment:

X **Previous experience by this PI. Please describe and cite references here or as an attachment.**

Some examples of studies that use 2 macaque monkeys:

Taylor DM, Helms Tillery SI, and Schwartz AB, Direct Cortical Control of 3D Neuroprosthetic Devices, Science 296: 1829-1832 (2002)

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Santhanam G, Ryu SI, Yu BM, Afshar A, Shenoy KV (2006) A high-performance brain-computer interface. Nature. 442:195-198.

Ganguly K. and Carmena J.M. (2009). Emergence of a stable cortical map for neuroprosthetic control. PLoS Biology 7(7): e1000153. doi:10.1371/journal.pbio.1000153

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Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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* * * Procedures * * *

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Positioner implantation
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	30	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

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Animal Utilization Proposal Form

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Approval Period: 09/19/2019-05/31/2021

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PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 15

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Procedure Description * * *

Procedure Description

Surgery preparations: Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.

The animal is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in stereotax and final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.

After sterile draping, the skin is incised along the midline from the orbital ridge to the occipital ridge with a #10 scalpel blade. The skin, muscle and fascia are reflected from an approximately 4-5 cm diameter area of the calvarium. Blunt scissors and a bone chisel are used to dissect and reflect the fascia and muscle respectively. The surface of the skull is cleaned with sterile saline. Hemostasis is achieved with gentle pressure with cotton tipped applicators or gauze pads. Tissues are moistened with sterile saline throughout the procedure to maintain viability and aid in healing. Suction is used to remove any excess saline and increase visibility in the surgical field. The titanium post (weight=10g) is positioned on the calvarium and the radial straps secured using titanium orthopedic screws. Small holes are made using an orthopedic hand-drill, then tapped using an orthopedic tap. The head positioner is finally secured by screwing the titanium, orthopedic screws into the tapped holes. The skin and subcutaneous tissue layers will be sewn completely over the orthopedic hardware (except for a small opening for the positioner itself) using 3-0 or 4-0 sutures. The positioner is intended to be permanent, but if necessary it can be removed in a separate procedure. The positioner will be placed no less than five weeks prior to the microwire array surgery.

Any deviation from any of the procedures or limits discussed here is only to be done in consultation with and with approval of OLAC veterinary staff and the ACUC.

How does this procedure fit into or address your overall research goals?

The head positioner enables us to fix the animals head in position. This enables us to perform eye tracking and to enable neurophysiological recording. This enables us to correlate neural activity to behavioral and cognitive processes.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff. Euthanasia would be considered in cases where the animal's condition did not respond to pain medication and/or clinical treatments.

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg).	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~1cm long) of ointment is applied to each eye during surgical prep. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	May be administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam.
Tramadol	3-5 mg/kg	Oral (PO)	Administered as a supplement to or a replacement for the injectable analgesic buprenorphine once the animal is eating reliably post-op.
Meloxicam	0.1mg/kg	Oral (PO)	Once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Imaging

Procedure Type:	Imaging	**Procedure Title:**	MRI scan in macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C

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NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Maximum number of animals to be used in this procedure for a THREE-YEAR period: 30

Was a veterinarian consulted (for D or E studies)?:

Use Location: [redacted]

Building Name: [redacted]

Room Number: [redacted]

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* * * Procedure Description * * *

Procedure Description

Magnetic Resonance Imaging (MRI)

There are substantial interindividual differences between macaques in the size of the brain and its precise layout. Therefore at least one MRI must be performed before implantation in order to know where specific brain structures are in stereotactic space. MRI images are necessary to accurately locate place the recording implants and for the construction of the skull model to enable manufacture of semichronic implants. These scans will be obtained on the 3T magnet in the [redacted]. Details regarding the procedure for obtaining MRI scans, including anesthetic induction, are included in a separate SOP.

An MRI scan is required to construct a 3D printed skull model that we will then use to ensure the accurate surgical placement of our recording electrodes. A further scan may be necessary to verify that the electrodes are in the correct position following implantation. (Electrodes are not in the brain during scanning - instead a contrast agent, such as Vitamin E, is placed in the recording grid which enables us to extrapolate the trajectory that the electrode would take into the brain). Additional scans may be required, for example, if scanning quality is poor. Consequently, we may scan an animal up to five times total. Because the animal must be anesthetized for each scan, a scan will not be performed within two weeks of a previous anesthetic event. This interval may be extended should there be concerns about the animal's health (e.g. loss of more than 10% of the animal's body weight).

The absolute decibel limit of the magnet is 102 dB, based on the fastest rise time the scanner can do. Most sequences are significantly lower than that, with maximum peaks around 85-90 dB (Hurwitz R, Lane SR, Bell RA, Brant-Zawadzki MN. Acoustic analysis of gradient-coil noise in MR imaging. Radiology 1989;173:545-548). We will use hearing protection (ear muffs or ear plugs) which can reduce noise up to 30dB.

How does this procedure fit into or address your overall research goals?

MRI scans ensure that our recording electrodes are positioned in the correct brain regions.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Describe post procedure monitoring that will be performed.

See attached MRI SOP.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Anesthetic Regimen * * *

X Respiratory Rate
X Heart Rate
X Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC is responsible for the anesthesia and anesthesia recordkeeping during this procedure.

Anesthetic Agents

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Ketamine hydrochloride	2--3mg/kg	Intramuscularly (IM)
Dexmedetomidine	0.015--0.04 mg/kg	Intramuscularly (IM)
Midazolam	0.25mg/kg	Intramuscularly (IM)
Isoflurane	1.5%-3.0%	Inhalation (IN)

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Atipamezole	0.15mg/kg	Intramuscularly (IM)	Once to reverse demedetomidine if necessary.

Behavior Study Assay

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Cognitive testing
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	30	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

The animals are trained to perform simplified ‘video games’ that test various aspects of cognitive performance in order to obtain rewards.

Training is performed using operant conditioning techniques. Whenever the animal makes a correct response

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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they will receive a reward; the animal then quickly learns to repeat this response to get another reward. The animal is seated facing a video display upon which visual cues are presented. The animal's task is to respond to the cue though arm movements, eye movements, or button presses. Some task examples are; 1) Using a joystick to move a cursor to hit a target stimulus; 2) Reaching to target stimuli on a touch-screen panel; 3) Reaching to touch buttons directed by a cue; and 4) fixating the gaze on a point on the screen for 2 seconds.

Training for each task varies depending on how the cue stimulus is delivered, how the animal is to interact and how the final goal/target is to be reached. The underlying theme for training is that each task is extremely easy at the beginning and becomes more specific and difficult as the animal learns how to earn the reward and becomes more proficient at performing the task. Training can continue for a period from one to several months, depending on the particular task required, the previous experience and inherent trainability of the animal, and the skill of the trainer. Animals are taught to perform the behavioral experiments required under this protocol, though the speed of training varies. Typically the length of the study varies from 3 months after surgery to several years in the case of the animal maintaining a stable population of neurons in the arrays of microelectrodes.

Cognitive testing typically occurs for 2 hours a day on a daily basis (maximum 3 hours). Our behavioral tasks are very sensitive assays to an animal's condition. If they are irritated for any reason, they stop working or do not perform as well as they do when not irritated. If there is any drop in behavioral performance, we always investigate potential causes of that drop. During neural recordings, animals are often out of the cage for 6 hours. This reflects the extra time for setting up the recording equipment, but cognitive testing still takes 2 hours (maximum 3 hours).

Cognitive testing will sometimes occur using an in-cage system, which has the advantage that the animal does not have to be handled, and also provides the animal with environmental enrichment. Liquid rewards will be tracked individually for each subject, either by separating the animals during the enrichment period, and eventually by using facial recognition software. At the end of testing, animals will receive an additional allotment if fluid if necessary. The number of hours per day that the device is available will depend on how animals wish to interact with the device. Some animals may prefer to work with it intensively, while others may prefer a more piecemeal approach. The device will remain in place permanently, but will only be activated when we wish to test the animal. We anticipate the device being available 5-7 days a week.

How does this procedure fit into or address your overall research goals?

Cognitive tests enable us to manipulate cognitive processes so that we can correlate neural activity to the appropriate cognitive process of interest.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Surgical Procedure

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Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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Procedure Type:	Surgical Procedure	**Procedure Title:**	Chronic microwire arrays implantation
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	16	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 15

Protocol Title: [redacted]

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* * * Procedure Description * * *

Procedure Description

Chronic microwire arrays implantation in macaques

The microwire arrays consist of biologically compatible, chronic microwire assemblies in array form (CD Neural Engineering, Durham, NC), built to be either stationary or moveable. The moveable electrodes can be adjusted in increments of 50 microns. Due to the fact that the brain has no pain receptors, adjustments to these electrodes can be made while the animal is awake in the chair. The number of wires in the array, the materials used and the separation between wires may vary. In some surgeries, cannula electrodes may be used for recording from subcortical regions. Gas sterilization is used for the arrays of microwires.

Surgery preparations: Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.

On the day of the surgery, the monkey is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in stereotax and final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.

Craniectomies: The skin is incised along the midline from the orbital ridge to the occipital ridge with a #10 scalpel blade. The skin, muscle and fascia are reflected from an approximately 4-5 cm diameter area of the calvarium. Blunt scissors and a bone chisel are used to dissect and reflect the fascia and muscle respectively. The surface of the skull is cleaned with sterile saline. Hemostasis is achieved with gentle pressure with cotton tipped applicators or gauze pads. Tissues are moistened with sterile saline throughout the procedure to maintain viability and aid in healing. Suction is used to remove any excess saline and increase visibility in the surgical field. 15-20 self-tapping screws, which are designed to not penetrate the cranium, are inserted into the skull. These screws play two important roles: to sustain the acrylic cap and to provide multiple ground points and hence maximize recording quality. A series of craniectomies are created in the skull at precise coordinates, measured stereotaxically, for the microwire electrode arrays. The maximum number of craniectomies per animal will be 10, 5 per hemisphere. A variety of drills may be used, including a dental drill and/or dremmel. Craniectomies usually range from 3X4mm to 5X8mm. As individual animals vary slightly from the standard stereotaxic map, craniectomies may be modified to better access a cortical and/or subcortical area. A given craniectomy may be extended for the purposes of localizing important landmarks on the surface of the brain, such as the central sulcus or the arcuate sulcus. Standard physiological localization is performed, using either tactile stimulation in a stroking pattern on the animal’s limb while searching for receptive fields in somatosensory areas, or by using surface macro-stimulation of cortical motor areas that evokes muscle activation in the animal’s limb. In addition, MRI images will be obtained before surgery for precise localization of the recording implants as well as for the construction of the skull model to enable manufacture of semichronic implants (see

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

'MRI scan in macaques' procedure and separate SOP). Bleeding is controlled with Gelfoam soaked in Thrombin or sterile saline. After the craniectomies have been created, the dura of each craniectomy is dissected.

Microwire Array Insertion: The arrays are positioned above the brain and lowered stereotaxically with a micromanipulator. As the wires make contact with the surface of the brain, a "zero" level is established, from which the depth of implantation is measured. As the arrays are lowered, qualified laboratory members monitor neuronal electrical activity detected by the microwires. Any noise interference from the room will be resolved to determine the array depth that records peak neuronal activity. A significant amount of noise comes from the various monitors used during surgery, including ECG and pulse oximetry, for example. A variety of strategies are employed sequentially to minimize noise such that recordings are acceptable. First, attempts are made to ground all equipment to the electrodes using sterile wires. Further noise may be resolved by putting electrical equipment in the room on battery operation for up to 20 minutes per implant. In extreme cases, all equipment using A/C electricity, except ECG, will be turned off for approximately 10 minutes to eliminate noise and improve recordings. Array depth is usually best between 1.5-2.5mm. Gel glue, liquid glue and glue accelerator may be used to initially secure the array, none of which can harbor biological contaminants. After each array is glued in position, the site is covered with acrylic up to the connector. The acrylic is applied in layers of liquid. As each layer dries quickly, additional layers can be applied at a reasonable rate. After the last connector is stabilized with acrylic, the exposed skull is covered with acrylic and acrylic is built up in between the connectors to stabilize all of them into the resulting acrylic cap. If it is decided during surgery that a craniectomy will not be used for inserting a microwire array, glue will be applied and acrylic will cover the craniectomy, just as for the others with arrays.

Acrylic Cap: The acrylic cap is made smooth where it may come in contact with the skin. 2 small, stainless steel nuts and a small, stainless steel pin will be embedded in the top of the acrylic cap for securing a protective layer above the connectors. Caps have an oval shape with maximum dimensions of 10cm along the anterior-posterior axis, and 8cm along the interaural axis. Approximate weight of the cap including stainless steel nuts is 100 grams. If the skin around the acrylic is loose or if bone is exposed, the skin is sutured using 3-0 or 4-0 sutures. Sutures are inspected daily and removed after 10-14 days. A topical antibiotic is applied to the skin at the border of the new acrylic. The wound is checked daily for signs of infection and/or bleeding. If necessary, the wound is cleaned with hydrogen peroxide and a topical antibiotic is applied. A light, protective cap is custom built to fit over the new acrylic headcap to keep the electrodes and the connectors clean. Any deviation from any of the procedures or limits discussed here is only to be done in consultation with and with approval of OLAC veterinary staff and the ACUC.

Long-term Care for Acrylic Cap Margin: The cap margin will be cleaned as necessary, up to three times weekly as documented in a cleaning log kept together with the animal's weight and water intake log. No anesthesia should be required for this cleaning procedure, although the animal will be monitored for any unanticipated distress. The margin is cleaned by the lab member in charge of the animal as follows: The entire wound edge is washed with chlorheximine (e.g. Nolvasan) scrub removing all scabs and crusts. Gauze soaked in Nolvasan solution is then placed over the entire circumference of the cap and left in place during the recording or training session or as long as possible. Fur is trimmed as needed. Infections are quite rare using these procedures, but if they do occur appropriate topical and/or systemic treatments are administered in consultation with OLAC veterinary staff.

How does this procedure fit into or address your overall research goals?

Chronic microwire arrays enable us to record neural activity across time (days to weeks) which enables us to correlate that activity with cognitive processes such as learning.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg)	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intravenous (IV)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~ 1 cm long) of ointment is applied to each eye during surgical preparation. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.
Fentanyl	2-50 mcg/kg/hour	Intravenous (IV)	It may be used adjunctively to lower the gas anesthetic requirement during surgery.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably take meds orally.
Tramadol	3-5 mg/kg	Oral (PO)	1-3 times daily up to 3 days.
Meloxicam	0.1mg/kg	Oral (PO)	Administered once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Behavior Study Assay

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Acclimation to restraint
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	30	**Was a veterinarian consulted (for D or E studies)?:**	

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NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Use Location: [redacted]
Building Name: [redacted]
Room Number: [redacted]

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* * * Procedure Description * * *

Procedure Description

Each animal is initially trained to be handled with the pole and collar method and to sit in a specially designed chair that permits both limb movements and postural adjustments. Once the animal is comfortable sitting in the chair, it is transported to the experimental room where it is trained to carry out progressively more complex behavioral tasks for a liquid reward. During task training, all trials are initiated by the animal, and it may generally work for as long as it wishes in a single session.

Animals will receive a substantial period of training (at least 1 month) in the chair before undergoing head positioner implantation and/or chronic implant surgical procedures. To condition animals to any head restraint that may be used, session duration is gradually increased from very short to normal length sessions. Head restraint, if used, will consist of the chair fixation post attached to the animal's headpost (see Positioner Implantation procedure). Training sessions last from 5 minutes to 6 hours, and are conducted from 0-7 days/week. During behavioral training, it is rarely necessary for head restraint to be greater than 3-4 hours per day. During electrophysiological recording, however, it is sometimes necessary to extend the period of head restraint up to (but not beyond) 8 hours per day. This provides us with sufficient time to troubleshoot problems with recording equipment if it should arise. Because of the length of head restraint, the chair is adjusted each day for maximum comfort, and the animal is free to move its limbs and adjust its posture in the chair. In addition, tasks have been designed to minimize stress on the animal by giving it as much control over its behavior as possible. The behavioral tasks consist of many short (typically <5-s) trials each of which, if performed successfully, rewards the animal with fluid. Animals become quite used to the laboratory environment. When animals get tired or have had enough fluid, they simply stop performing the task, in which case they will be returned to their home cage.

Training is performed using operant conditioning techniques. Whenever the animal makes a correct response they will receive a reward; the animal then quickly learns to repeat this response to get another reward. In addition, clicker training is used for positive reinforcement.

See "Acclimation to Restraint" attachment for training summary.

How does this procedure fit into or address your overall research goals?

Restraint enables us to track eye movements and to record neural activity, enabling us to correlate neural activity with ongoing behavioral and cognitive processes.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Chamber placement for semichronic arrays or acute recordings in macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	30	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any

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Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 15

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Procedure Description * * *

Procedure Description

Acute recordings involve lowering electrodes into the brain each day and then removing them at the end of the session. In contrast, in semichronic recordings, although the electrode position remains adjustable, the electrodes remain in the animal's brain for several months. Both types of recording require chambers to enable access to the animal's brain and to control the position of the electrodes.

Each animal will have up to four recording chambers depending on the experiment. This enables us to record simultaneously from multiple brain areas in order to determine the flow of information through the brain during the performance of the behavioral task. All the chambers will be placed at the same time. The sites of the chambers are determined stereotactically from the MRI scans to ensure that we hit the brain sites of interest for the experimental aims. This procedure has been developed and refined over the last two decades and is in use in multiple neurophysiological laboratories.

Surgery preparations

Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water. The animal is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for preemptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head fur is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivacaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in the stereotax and the final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.

Surgical procedure

After sterile draping, the skin is incised along the midline with a #10 scalpel blade. The skin, muscle and fascia are reflected using blunt scissors and a bone chisel. The surface of the skull is cleaned with sterile saline. Hemostasis is achieved with gentle pressure with cotton tipped applicators or gauze pads. Tissues are moistened with sterile saline throughout the procedure to maintain viability and aid in healing. Suction is used to remove any excess saline and increase visibility in the surgical field. The chamber is positioned on the calvarium and attached using bone cement and titanium orthopedic screws. The location of the chamber is determined by stereotactic coordinates (Paxinos G, Huang X-F, Toga A (2000) The rhesus monkey brain in stereotaxic coordinates. San Diego (California): Academic Press). Small holes are made using an orthopedic hand-drill, and then tapped using an orthopedic tap. In the case of the implantation of semichronic arrays, a tight seal is ensured between the cranium and the chamber by using MR images (see 'MRI Scan in Macaques' procedure) to construct a custom recording chamber that is shaped to the contours of the skull. Metabond bone cement will be used to create a water-tight seal at the interface between the cranial bone and chamber.

Chamber size will vary from 1.7 cm – 2.7 cm across, and 1.0 – 3.2 cm height from the midpoint of the chamber. The weight depends on the chamber size, with a maximum weight of 50g. Bone cement (Palacos, a

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

methylmethacrylate methylacrylate copolymer) is applied in thin fluid layers directly to the perimeter of the base of the chamber where it comes in contact with bone. Once the initial layer has cured (20 sec – 5 mins), a second thin fluid layer will be applied around the perimeter of the chamber and bone screws positioned around the chamber. Additional bone cement is applied, layer by layer, until a cap has been formed. The bone cement is built up to the bottom lip of the chamber to ensure appropriate structural integrity of the chamber. The acrylic cap is made smooth where it may come in contact with the skin. Caps have an oval shape with maximum dimensions of 10cm along the anterior-posterior axis, and 8cm along the interaural axis, and an approximate maximum weight of 100 grams. The skin and subcutaneous tissue layers will be sewn around the cap using 3-0 or 4-0 sutures.

Long term care of skin margin

The cap margin will be cleaned when the animal is taken out of the cage to perform tasks. The cap margin will be cleaned as necessary, up to three times weekly as documented in a cleaning log kept together with the animal's weight and water intake log. The margin is cleaned by the lab member in charge of the animal as follows: The entire wound edge is washed with chlorhexidine solution (e.g. Nolvasan) scrub removing all scabs and crusts. Gauze soaked in Nolvasan solution is then placed over the entire circumference of the cap and left in place during the recording or training session or as long as possible. Fur is trimmed as needed. Infections are quite rare using these procedures, but if they do occur appropriate topical and/or systemic treatments are administered in consultation with OLAC veterinary staff. Typically these may include a topical antibiotic (e.g. Elase Chloromycetin gel, Nitrofurazone or an alternative agent), topical Chloramphenicol (or an alternative agent), or a systemic antibiotic (e.g. Cephalexin or an alternative agent).

Long term care of cranial implants

Chambers are maintained per APV Guidelines (attached) in terms of Routine Recording Cylinder Care or in consultation with an OLAC veterinarian.

Repositioning of chambers

On occasion, the position of the chambers may need to be adjusted, should they prove unsuitable for accessing the required areas. This might occur for a number of reasons. First, there is the inherent inaccuracy in placing the chambers. Small differences in the angle of the chamber can have large differences in the final position of the electrodes, particularly when one is aiming for structures that are only several millimeters in size. Second, neurons encoding selective information may be detected on the edge of the area of accessible brain. Third, new information might arise in the scientific literature indicating that another brain structure might be important for the cognitive process that the behavioral task taxes. Fourth, after extended recording from the same location there can be difficulty obtaining viable neurons, necessitating recording from the opposite hemisphere. Fifth, we occasionally detect laterality effects (since we record from opposite hemispheres in the two animals that we use for each study) which may necessitate recording from the opposite hemisphere. Finally, there is sometimes breakdown of integrity of the acrylic cap necessitating removal and re-implantation at a later date. Any of these reasons could necessitate a change in position of the recording chamber. Each of these reasons is infrequent and it is unlikely that all will occur in a single subject. However, taken together it is likely that all subjects will at some point need the chambers repositioned at least once, at a maximum of one reposition per year, and a maximum of 3 per animal. Adjustment of the chamber position will take place in a single surgery. First, the old chamber and cap will be removed using drills and rongeurs to remove overlying bone cement, and then we unscrew the orthopedic screws. Then a new chamber will be attached as described above. Any deviation from any of the procedures or limits discussed here is only to be done in consultation with and with approval of OLAC veterinary staff and the ACUC.

How does this procedure fit into or address your overall research goals?

Recording chambers provide us with a means to access the brain through a craniotomy and record neural

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

activity.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

*** * * Surgeon Details * * ***

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg).	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~1cm long) of ointment is applied to each eye during surgical prep. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	May be administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam.
Tramadol	3-5 mg/kg	Oral (PO)	Administered as a supplement to or a replacement for the injectable analgesic buprenorphine once the animal is eating reliably post-op.
Meloxicam	0.1mg/kg	Oral (PO)	Once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Behavior Study Assay

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Neural recordings and microstimulation using semichronic arrays in macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Maximum number of animals to be used in this procedure for a THREE-YEAR period: 30

Was a veterinarian consulted (for D or E studies)?:

Use Location: [redacted]

Building Name: [redacted]

Room Number: [redacted]

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* * * Procedure Description * * *

Procedure Description

One problem with chronic microarrays is that over time recording quality diminishes as gliosis occurs around the electrode contacts. Semichronic arrays provide a potential solution to this problem. The implant assembly is similar to that used for acute recordings. However, the electrodes and microdrives are designed to remain in place chronically. Thus, although the implant is chronic, individual electrodes remain adjustable. Therefore, if gliosis occurs the electrode can be simply advanced to establish new neuronal recordings. The leading manufacturer of these devices is Gray Matter Research Inc. [1]. The number of implanted electrodes varies depending on the specific study (range: 32-256 electrodes). A fraction of these electrodes will target cortical areas whereas other electrodes will target subcortical areas. Note: 256 is a typical number in chronic implant procedures (see for example: Ganguly K. and Carmena J.M. (2009) Emergence of a stable cortical map for neuroprosthetic control. PLoS Biology 7(7): e1000153. doi:10.1371/journal.pbio.1000153).

The electrodes encased inside the microdrive assembly are able to be lowered into the brain and have their positions adjusted over time. Sterility of the electrodes is ensured via a silicone barrier that sterile electrodes must pierce in order to enter the brain. Due to this barrier, cleaning is not required for up to 12 months as the chambers remain completely sealed during this time. As electrodes are inserted into the brain, signals are monitored during the initial electrode lowering until all electrode tips are past the dura and have entered cortex. The final depth of the electrode varies from 2mm in cortical structures on the surface (e.g. dorsolateral prefrontal cortex) to 40mm in deep cortical structures (e.g. ventromedial prefrontal cortex). The precise electrode positions will be adjusted on that day using the microdrives until the maximum number of electrodes yield high quality neuronal recordings. At this time, the electrodes will remain in the brain for a period of a minimum of several weeks, but often as long as six to twelve months. During this time, micro adjustments in electrode position (using the microdrives) will allow for optimal electrophysiological recordings --and the microdrive adjustment is achieved while maintaining the tight seal of the system.

At the end of each experimental session, the chamber system is covered with a titanium cap. No cleaning is required at the end of a recording session due to the sealed and enclosed design of the system. If microelectrodes need to be replaced (or upon completion of the experiment and recordings), all electrodes and the microdrive system will be removed as per the procedure outlined in Calvarium Opening for Semichronic Arrays in Macaques. Currently, there have not been any reported complications or infection/inflammation to the tissue in the Gray Matter recording chamber system (personal communication with [redacted] at the [redacted], [redacted] at [redacted] and [redacted] at the [redacted]).
Direct electrical stimulation into the brain may be used as a way of providing feedback (e.g. stimulation applied to somatosensory areas can be used to improve motor control), to entrain neural networks (e.g. patterned stimulation can be used to produce desired patterns of neural activity), and to change behavior as a causal intervention (e.g. stimulating reward areas to ensure behaviors are repeated). For example, preferences for particular actions (e.g. choosing between two targets on a screen) are encoded in patterns of neural activity and this encoding can be altered using stimulation due to the fact that neurons communicate to each other through

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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electrical signaling. Electrical stimulation is safe tool widely used by the electrophysiology community [2-6]. Different spatiotemporal patterns are applied in specific electrodes of the semichronic array using biphasic stimulation. Current is applied up to 300uA, with a resulting voltage of –10V to 10V, a maximum frequency of 300Hz and a maximum duration of 1000ms [4 - 6].

[1] http://www.graymatter-research.com/
[2] K. Nakamura and O. Hikosaka. Facilitation of saccadic eye movements by postsaccadic electrical stimulation in the primate caudate. J. Neurosci. 26(50), 12885 – 12895 (2006).
[3] T.D. Hanks, J. Ditterich, M.N. Shadlen. Microstimulation of macaque area LIP affects decisionmaking in a motion discrimination task. Nat. Neurosci. 9(5), 682 – 689 (2006).
[4] Z.M. Williams and E.N. Eskandar. Selective enhancement of associative learning by microstimulation of the anterior caudate. Nat. Neurosci. 9(4), 562 – 568 (2006).
[5] K. Amemore and A.N. Graybiel. Localized microstimulation of primate pregenual cingulate cortex induces negative decision-making. Nat. Neurosci. 15(2), 776 – 787 (2012).
[6] S.A. Overduin, A. d'Avella, J.M. Carmena and E. Bizzi. Microstimulation activates a handful of muscle synergies. Neuron 76(6), 1071-1077 (2012).

How does this procedure fit into or address your overall research goals?

The overarching goal of this project is to understand the relationship between neural activity and cognitive and behavioral processes. We use both correlative (measurement of neural activity) and causative (electrical stimulation of neurons) in order to accomplish this.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Chlorhexidine	0.05%	Irrigation	If microelectrodes need to be replaced all electrodes and the microdrive system will be removed so the chamber can be thoroughly irrigated with sterile saline and Nolvasan

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Calvarium opening for semichronic and acute arrays in macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	30	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 15

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Procedure Description * * *

Procedure Description

Acute recordings involve lowering electrodes into the brain each day and then removing them at the end of the session. In contrast, in semichronic recordings, although the electrode position remains adjustable, the electrodes remain in the animal's brain for several months. Both types of recording require chambers to enable access to the animal's brain and to control the position of the electrodes.

After the chamber placement procedure, a separate surgery is performed at a later date (typically between 1-2 weeks) to make an opening in the calvarium. The underlying dura is left intact or slightly reduced using suction to remove any granular tissue. This is a minor procedure since the periosteum (which contains sensory nerves) has already been removed, and because there is no dissection of the dura. Thus, the animals typically recover extremely rapidly from this procedure. The animal is returned to a restricted water schedule when they are bright, alert, and responsive, and have a normal appetite. This enables us to begin recording soon after the opening has been constructed, and thus when little granulation tissue has formed, increasing the quality of the data.

Surgery preparations: Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.

On the day of the surgery, the monkey is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head fur is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in the stereotax and the final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures. To make the opening in the calvarium, a variety of drills may be used, including a dental drill and/or dremel. The underlying dura is left intact or slightly reduced, using suction to remove any granular tissue. Bleeding is controlled with Gelfoam soaked in Thrombin or sterile saline.

On occasion, the position of the chambers may need to be adjusted, should they prove unsuitable for accessing the required areas. This might occur for a number of reasons. First, there is the inherent inaccuracy in placing the chambers. Small differences in the angle of the chamber can have large differences in the final position of the electrodes, particularly when one is aiming for structures that are only several millimeters in size. Second, neurons encoding selective information may be detected on the edge of the area of accessible brain. Third, new information might arise in the scientific literature indicating that another brain structure might be important for the cognitive process that the behavioral task taxes. Fourth, after extended recording from the same location there can be difficulty obtaining viable neurons, necessitating recording from the opposite hemisphere. Fifth, we occasionally detect laterality effects (since we record from opposite hemispheres in the two animals that we use for each study) which may necessitate recording from the opposite hemisphere. Finally, there is sometimes breakdown of integrity of the acrylic cap necessitating removal and re-implantation at a later date.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Any of these reasons could necessitate a change in position of the recording chamber. Each of these reasons is infrequent and it is unlikely that all will occur in a single subject. However, taken together it is likely that all subjects will at some point need the chambers repositioned at least once, at a maximum of one reposition per year, and a maximum of 3 per animal. Adjustment of the chamber position will take place in a single surgery. First, the old chamber and acrylic will be removed using drills and rongeurs to remove overlying acrylic, and then we unscrew the orthopedic screws. Then a new chamber will be attached (see “Chamber Placement for Semichronic Arrays” procedure). One to two weeks after this surgery a new craniotomy will be performed.

Chambers are also sometimes removed for clinical reasons, such as the chamber becoming loose or an infection developing underneath the implant. In addition, there can be some growth of both granulation tissue and bone inside the chamber, although this varies considerably between individual animals. In such cases it may be necessary to perform a surgical procedure to reduce the dura to remove the additional bone and granulation tissue. Explantations for clinical purposes as well as dura reductions are not included in the limit of 3 per animal.

How does this procedure fit into or address your overall research goals?

The calvarium opening enables us to acces the brain so as to record neural activity.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Midazolam	0.1--0.25mg/kg	Intravenous (IV)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~ 1 cm long) of ointment is applied to each eye during surgical preparation. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam.
Tramadol	3-5 mg/kg	Oral (PO)	1-3 times daily up to 3 days.
Meloxicam	0.1mg/kg	Oral (PO)	Administered once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

*** * * Other Agents Utilized * * ***

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Dural maintenance
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	30	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 15

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Procedure Description * * *

Procedure Description

On occasion it is necessary to remove granulation tissue and scar tissue from the dural surface. This arises when the tissue begins to prevent us from inserting our electrodes into the brain. During acute recordings, this occurs approximately every 4-8 weeks. Thus, in a typical recording session an animal will undergo one surgery to maintain the dura. The maximum number of times an animal would require dural maintenance is 6. The minimum time between procedures would be 2 weeks. This would occur if the first procedure was not successful in thinning the dura.

Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.

On the day of the surgery, the monkey is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head fur is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in the stereotax and the final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures. The surgical procedure itself is straightforward: we use aspiration with micropipettes to remove extraneous tissue.

How does this procedure fit into or address your overall research goals?

We need to introduce electrodes into the brain in order to record neural activity. In order to record the activity of single neurons, the tips of these electrodes are extremely fine (~1 um) and can be damaged if the dura is thickened, as can happen when the dura is exposed. Dural maintenance keeps the dura thin enough to allow the electrodes to be inserted without damage.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg)	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intravenous (IV)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~ 1 cm long) of ointment is applied to each eye during surgical preparation. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam
Tramadol	3-5 mg/kg	Oral (PO)	1-3 times daily up to 3 days.
Meloxicam	0.1mg/kg	Oral (PO)	Administered once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

*** * * Other Agents Utilized * * ***

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Electrocorticographic (ECoG) arrays implantation
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	16	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]

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NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Room Number: [redacted]

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 15

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Procedure Description * * *

Procedure Description

Implantation of electrocorticographic (ECoG) arrays in macaques

Electrocorticographic (ECoG) arrays consist of biologically compatible electrode contacts embedded in a plastic polymer sheet (Neuronexus, Ann Arbor, MI), designed to record electrical potentials from the surface of the cortex. Additional details regarding the arrays and their surgical implantation have been reported in a methodological paper (Fukushima et al. 2014).
There are three main reasons to use ECoG arrays:
i). They are directly analogous to the implants that are being implanted in patients thereby improving the translational relevance of our findings.
ii). They enable us to record a different neural measure. Because we don't understand how cognitive processes are instantiated in the brain, it behooves us to record neural signals at a variety of different scales (single-unit, local field potentials, surface potentials).
iii). They enable us to cover a much larger cortical surface, with the aim of understanding the topographic organization of a given cognitive process.
The number of contacts in the array, the materials used and the separation between contacts may vary. Gas sterilization is used for the arrays. The total size of the array is typically about 1 cm2 and the total weight of the implant will be approximately 5g.

Surgery preparations: Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.
On the day of the surgery, the monkey is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Mannitol (typically 2.0 g / kg i.v. 30 minutes prior to the procedure) may be administered to counteract potential cerebral edema.
Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in stereotax and final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.
Implantation: The skin is incised along the midline from the orbital ridge to the occipital ridge with a #10 scalpel blade. The skin, muscle and fascia are reflected from an approximately 4-5 cm diameter area of the calvarium. Blunt scissors and a bone chisel are used to dissect and reflect the fascia and muscle respectively. The surface of the skull is cleaned with sterile saline. Hemostasis is achieved with gentle pressure with cotton tipped applicators or gauze pads. Tissues are moistened with sterile saline throughout the procedure to maintain viability and aid in healing. Suction is used to remove any excess saline and increase visibility in the surgical field. A bone flap is created in the skull. A variety of drills may be used, including a dental drill and/or dremmel. The bone flap will be approximately 4 cm x 3 cm, with the long edge running approximately 1 cm parallel to the midline, with the short edge abutting the brow ridge. The bone flap is maintained in sterile saline for the remainder of the surgery until it needs to be sutured back in place. A dural resection is then performed. The dura

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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is carefully pulled away from the surface of the brain using fine forceps, and a cut is made using a microdissecting hook. A T-shaped incision is then made using a size 15 scalpel. The resection will be large enough to allow the implantation of the array i.e. uncovering about 2 cm2 of cortex. The array is positioned on the surface of the dura using either stereotactic co-ordinates or sulcal landmarks. The dura is then sutured over the top of the array using 5-0 absorbable suture, with a small opening for the array leads. Four holes are drilled through the skull about 5 mm from the dorsal and posterior edge of the bone flap and 3-0 absorbable suture is threaded through each hole. A grooved brain spatula is positioned under the skull to prevent damage to the dura during this process. Holes are drilled in corresponding locations in the bone flap itself, and the sutures are used to suture the bone flap in place. The leads are fed through the edge of the flap. The leads and connector are secured to the skull posteriorly to the bone flap using bone cement and titanium screws.
The above procedures apply to any placement of the array on the dorsal or lateral surface of the brain. The procedures are slightly different for a ventral placement. A larger bone flap is made by extending the flap medially to the midline and laterally to about the base of the frontal bone. This flap is approximately 4 cm x 5 cm. The part of the brow ridge covering the very tip of the frontal pole is then resected using rongeurs so as to ensure the brain is not damaged when elevated. The stereotax arms are then rotated about 30°, allowing gravity to help create a space between the brain and the orbital surface of the skull. A large dural resection is then performed, uncovering about 8 cm2 of cortex, in order to provide sufficient space to raise the frontal lobe. The ECoG array is slid under the surface of the brain using a brain spatula to elevate the brain. All other steps are the same as the dorsal/lateral placement.

Bone cement cap: The bone cement cap is made smooth where it may come in contact with the skin. 2 small, stainless steel nuts and a small, stainless steel pin will be embedded in the top of the acrylic cap for securing a protective layer above the connectors. Caps have an oval shape with approximate dimensions of 3-cm along the anterior-posterior axis, and 2-cm along the interaural axis. The skin is sutured around the cap and over the top of the bone flap using 3-0 or 4-0 absorbable suture. The wound margin is checked daily for signs of infection and/or bleeding. If necessary, the wound is cleaned with chlorhexidine solution and/or dilute betadine solution and a topical antibiotic is applied. A light, protective cap is custom built to fit over the new headcap to keep the electrodes and the connectors clean. Any deviation from any of the procedures or limits discussed here is only to be done in consultation with and with approval of OLAC veterinary staff and the ACUC.
Long-term Care for Acrylic Cap Margin: The cap margin will be cleaned as necessary, up to three times weekly as documented in a cleaning log kept together with the animal's weight and water intake log. No anesthesia should be required for this cleaning procedure, although the animal will be monitored for any unanticipated distress. The margin is cleaned by the lab member in charge of the animal as follows: The entire wound edge is washed with chlorhexidine (e.g. Nolvasan) scrub removing all scabs and crusts. Gauze soaked in Nolvasan solution is then placed over the entire circumference of the cap and left in place during the recording or training session or as long as possible. Fur is trimmed as needed. Infections are quite rare using these procedures, but if they do occur appropriate topical and/or systemic treatments are administered in consultation with OLAC veterinary staff.

Fukushima, M., R. C. Saunders, M. Mullarkey, A. M. Doyle, M. Mishkin, and N. Fujii. 2014. 'An electrocorticographic electrode array for simultaneous recording from medial, lateral, and intrasulcal surface of the cortex in macaque monkeys', J Neurosci Methods, 233: 155-65.

How does this procedure fit into or address your overall research goals?

ECoG arrays allow us to collect neural activity across a wide expanse of brain area (~ 1 cm x 1 cm) which allows us to understand how information is organized across the cortical mantle.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	N	Neurosurgeon with 20+ years of NHP neurosurgery [redacted], NIH) will be responsible for performing initial surgeries and training [redacted]

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg)	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intravenous (IV)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~ 1 cm long) of ointment is applied to each eye during surgical preparation. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam.
Tramadol	3-5 mg/kg	Oral (PO)	1-3 times daily up to 3 days.
Meloxicam	0.1mg/kg	Oral (PO)	Administered once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Mannitol	1-2g/kg	Intravenous (IV)	1 dose 30 minutes prior to surgery

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Compound/Drug Administration

Procedure Type: Compound/Drug Administration

Procedure Title: Neuropharmacological manipulation during cognitive testing

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Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Species: Monkey, Rhesus (OLAC Vivarium)

Pain/Distress Category: C

Maximum number of animals to be used in this procedure for a THREE-YEAR period: 16

Was a veterinarian consulted (for D or E studies)?:

Use Location: [redacted]

Building Name: [redacted]

Room Number: [redacted]

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* * * Procedure Description * * *

Procedure Description

The aim of this technique is to assess the electrophysiological effects of pharmacologic compounds at the single cell level. Data obtained through use of this system can be used to directly compare neuronal firing rates, the incidence of bursts and other parameters of neuronal discharge at the single-cell level before, during, and after drug injections. Effects of these manipulations are assessed while the animals perform simplified 'video games' that test various aspects of cognitive performance in order to obtain rewards.

The injection system consists of silica tubing, stainless steel cannulae combined with a tungsten or platinum-iridium electrode (collectively referred to as an 'injectrode'). All solutions that are perfused through the system are filtered using a 0.250 µm micropore filter (Fisher Scientific, Hampton, NH) before use to remove any particles from being incorporated into the final injection solution, and to prevent contamination of the system. Flow through the injection system is established with repeated flushes with filtered distilled water at a rate 0.5–1.0 µl/min before being positioned into the microdrive. Once the system is fixed to the microdrive, one of the three liquid switch ports is connected to it, and from this point on until the end of the drug injection, there is continuous flow of either a CSF or drug solution through the injection system. The flow rate is very slow (0.1 µl/min) so the overall volume injected is minimal (~3 µl) but the continual positive pressure prevents clogging of the injection system. After use, the assembly is cleaned following the "fluid capillary loading and cleaning SOP".

There are two agents that are known to influence neuronal activity in prefrontal cortex that we may wish to infuse. All agents will be pharmaceutical grade. The precise dosages that we use have been determined from the existing literature, and we will use multiple concentrations to ensure that a reasonable proportion of the dose-response curve is measured. We wish to test an antagonist of the dopaminergic system (D1 antagonist SCH23390) and a GABA agonist (muscimol).

We do not anticipate any effect on behavior. Indeed, such an effect would be detrimental to the interpretation of our results, since we would not be sure whether the change in neuronal activity was a direct result of the drug on the neuron, or an indirect effect on neuronal activity because the drug has altered the animal's behavior. We do not anticipate there being any behavioral consequence, as the amount of drug necessary to produce a neuronal effect is typically an order of magnitude less than the amount necessary to produce a behavioral effect. For example, with respect to muscimol, 0.5 µg is sufficient to produce a neuronal effect (Kliem & Wichmann 2004), yet 10-20 µg is necessary to observe a behavioral effect (Amiez et al 2006, Shima & Tanji 1998). Each infusion will involve the injection of no greater than 1 µl of fluid, to limit spread through cortical tissue and reduce the likelihood of observing behavioral effects. If there were behavioral effects they would be subtle and only evident using an appropriate behavioral assay, since we are infusing the substances into prefrontal cortex. For example, we might expect working memory impairments or attentional deficits, but these could only be detected if the animal were performing a task that taxed these cognitive processes.

Dosages will be based on a combination of experimentation and guidance from the literature. For some agents, the dosage can be determined straightforwardly from their effects of neural activity. For example, muscimol

Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

the dosage can be determined straightforwardly from their effects of neural activity. For example, muscimol increases inhibitory activity and causes the cessation of pyramidal cell activity. Thus, we can confirm that 0.5 µg is the smallest dose that ensures this will happen. In other cases, the agent's effects on neural firing are complex and relate to the information that is encoded by the neurons. In this case, we will base the dosages on the least effective dose in producing a behavioral effect in the existing literature. We will begin with a dosage one order of magnitude lower than this dose. For example for the D1 antagonist SCH23390, where the least effective behavioral dose is 15 µg (Sawaguchi & Goldman-Rakic 1994), we would begin with a dosage of 1 µg, and then increase the dosage in 5 µg increments to 15 µg. In addition, the effects of the drugs are short lasting. Even at the highest doses tested of SCH23390 (60 µg), behavioral effects had disappeared after 60 minutes. In sum, we will minimize the chances of seeing behavioral effects by using small quantities and small concentrations of short-acting drugs. However, we note that this is an inexact science. For example, initial studies argued that Raclopride, a D2 antagonist, was behaviorally ineffective at doses as high as 100 µg (Sawaguchi & Goldman-Rakic 1994). However, later studies showed that D2 receptors encoded reward information (Wang et al 2004), while the behavioral assays had tested working memory. In other words, the behavioral ineffectiveness of Raclopride was because the wrong behavior had been tested, not because it was a weakly effective agent. To err on the side of caution, we will always begin our studies with low doses of drug (1 µg).
We do not anticipate any appreciable increase in the amount of mechanical neurological damage over and above a standard neurophysiological experiment. The injectrode is 210 µm in diameter, which is considerably smaller than the electrodes that we use in our neurophysiological experiments, which have a typical diameter of 300 µm. In addition, a typical experiment will require fewer penetrations than our neurophysiology experiments. A single study would contrast the effects of the two agents on about 30 neurons per animal, necessitating about 120 total infusions (30 neurons for each of the two agents, plus controls where vehicle alone is infused). We would perform one session per day, with a single session involving the lowering of up to two injectrodes per hemisphere. Thus, we anticipate making fewer than 120 penetrations with this system, per animal per experiment, whereas a neurophysiology experiment can require 400 penetrations. In sum, we expect this experiment to produce less mechanical damage than a neurophysiology experiment.

Amiez C, Joseph JP, Procyk E. 2006. Reward encoding in the monkey anterior cingulate cortex. Cereb Cortex 16: 1040-55
Kliem MA, Wichmann T. 2004. A method to record changes in local neuronal discharge in response to infusion of small drug quantities in awake monkeys. J Neurosci Methods 138: 45-9
Sawaguchi T, Goldman-Rakic PS. 1994. The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J Neurophysiol 71: 515-28
Shima K, Tanji J. 1998. Role for cingulate motor area cells in voluntary movement selection based on reward. Science 282: 1335-8
Wang M, Vijayraghavan S, Goldman-Rakic PS. 2004. Selective D2 receptor actions on the functional circuitry of working memory. Science 303: 853-6

How does this procedure fit into or address your overall research goals?

The aim of this technique is to assess the electrophysiological effects of pharmacologic compounds at the single cell level. Data obtained through use of this system can be used to directly compare neuronal firing rates, the incidence of bursts and other parameters of neuronal discharge at the single-cell level before, during, and after drug injections. Effects of these manipulations are assessed while the animals perform simplified 'video games' that test various aspects of cognitive performance in order to obtain rewards.This enables us to assess the effects of pharmacological compounds on neural coding in order to assay novel treatments for neuropsychiatric disorders that feature impaired cognitive processing.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Peri procedure Care/Analgesics * * *

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Post-procedure Monitoring

Recovery Location Building Name

Room Number

Responsible Personnel

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

Monitoring Duration

Monitoring Frequency

Describe what actions will be taken if parameters monitored fall outside normal ranges:

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Describe record keeping/documentation methods for post-procedure monitoring:

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
SCH23390	1-15 ug in 1 ul vehicle	Intracranial	Infused for 0-5 mins, 1-5 times a session
aCSF	1 ul	Intracranial	Infused for 0-5 mins, 1-5 times a session
Muscimol	0.1-10 ug	Intracranial	Infused for 0-5 mins, 1-5 times a session

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Protocol Title: [redacted]
Approval Period: 09/19/2019-05/31/2021
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* * * Alternative Search * * *

For Pain/Distress Categories D or E

For any procedure that is likely to cause more than slight or momentary pain or distress, a literature search is required to determine if other methods are available that could reduce or eliminate pain or distress experienced by the animal. Instructions and examples of this literature search, appropriate databases to use, and helpful keywords can be found in the guidelines on Literature Searches for Alternatives on the ACUC website.

Search Data

Search Date	Search Range	Keywords	Databases Searched
04/05/2018	1991-2018	Head restraint, positioner, head post, rhesus macaque	PUBMED/ Web of Science/ Society for Neuroscience Abstracts
04/05/2018	1991-2018	multielectrode recordings, rhesus macaque, cortex, brain-machine interface, computational model, computer simulations, non-invasive neuroprosthetics	PUBMED/ Web of Science/ Society for Neuroscience Abstracts
04/05/2018	1991-2018	rhesus macaque, cortex, motor control, motor learning, computational model, computer simulations, motor psychophysics	PUBMED/ Web of Science/ Society for Neuroscience Abstracts

Describe the search strategies used to conduct your search.

Methods other than a literature search:

Professional conferences/meetings attended: Society for Neuroscience annual meeting, CoSyNe, Computational Properties of Prefrontal Cortex, Bioengineering retreats, Helen Wills Neuroscience annual retreats.

Names of other experts consulted [redacted]

List of service provided to grant review committees, panels or editorial boards:Grant reviewer for the National Institutes of Health study section SPC, Grant reviewer for the National Institutes of Health study section NMB, Frequent reviewer for Nature, Nature Neuroscience, Science, PNAS, PLoS Biology, Journal of Neuroscience, Neuron, eLIFE, Journal of Neurophysiology, Cerebral Cortex and Journal of Cognitive Neuroscience

List of journals subscribed to and/or read: Science, Nature, J. Neuroscience, J. Neurophysiology, PNAS, PLoS Biology, Current Biology, Nature-Neuroscience, Neuron, J. Neuroscience Methods, Neuroreport. Newer searches also included the following journals: Experimental Brain Research; Brain; British Journal of Anesthesia;

List of seminars/lab meetings presented/attended (last 5 years): Princeton, AREADNE, Mt. Sinai, Gordon Research Conference, FENS, Johns Hopkins, UC Davis, International Symposium of the GRSNC, Neurobiology of LEarning and Memory, International Congress of Psychology, Oxford, Weizmann

Provide the number of hits and summarize the findings of your search results.

We undertook a literature search to examine whether there are any computational models that might explain the higher cognitive processes that we are studying. All of the models were either purely computational, or had only been validated at the level of gross function (blood flow

Protocol Title: [redacted])

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

through a brain region). No study has yet validated a computational model at the neuronal level. Indeed, we are currently collaborating with one of the creators of one of these models, in attempt to validate some of the model's claims.

We also conducted a literature search to examine whether a non-invasive method exists to measure single neuron activity. Several papers have recently explored the relationship between single neuron activity and functional magnetic resonance imaging. Rather than showing that we can use functional neuroimaging in place of single-unit physiology, they have instead emphasized the difference in the two signals. Thus, at present there is no other method for recording the activity of the activity of single neurons in the brain other than single unit neurophysiology.

Finally, we examined whether there were alternatives to the semi-chronic microarrays that we are proposing to use. Although the search term turned up several companies using microarrays (e.g. Blackrock and Microprobes) none of these implants incorporated the ability to adjust the electrode depth post-implantation, and consequently they will all suffer from the gradual degradation of signal that occurs in chronically implanted microarrays.

I believe there is no alternative to further reduce, replace or refine this potentially painful/distressful procedure. Based on the following references and experience, this animal model is the most appropriate for conducting my research.

Specific Relevant Citations

Relevant citations must be listed here or provided as an attachment.

Botvinick MM, Niv Y, Barto AC (2009) Hierarchically organized behavior and its neural foundations: a reinforcement learning perspective. Cognition 113:262-280.

Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A (2001) Neurophysiological investigation of the basis of the fMRI signal. Nature 412:150-157.

Mukamel R, Gelbard H, Arieli A, Hasson U, Fried I, Malach R (2005) Coupling between neuronal firing, field potentials, and FMRI in human auditory cortex. Science 309:951-954.

Sirotin YB, Das A (2009) Anticipatory haemodynamic signals in sensory cortex not predicted by local neuronal activity. Nature 457:475-479.

For category E procedures, explain why drugs or other ameliorative treatments cannot be used to fully alleviate pain/distress. Include the number of animals per year.

Protocol Title: [redacted])

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Procedure Relationships * * *

Procedure Relationships

Please describe the sequence and timing of the manipulations:

If more than one surgery or procedure will be performed on some or all animals used under this protocol, describe the sequence and timing of these manipulations. Flow charts may be helpful and can be attached to the protocol.

Animals are used in multiple experiments (sequentially) until recordings are not viable due to microelectrode malfunction and/or signal degradation.
Up to 30 macaques (with a maximum of 3 macaques per study but typically these will be 2) will be used in these experiments. Up to 5 studies may start in a given year. Each animal is initially trained to be handled with the pole and collar method and to sit in a customized chair. Once the animal is comfortable sitting in the chair, it is transported to the experimental room where it is trained to carry out progressively more complex behavioral tasks for a liquid reward. During task training, all trials are initiated by the animal, and it may generally work for as long as it wishes in a single session. Training sessions last from 5 minutes to 6 hours, and are conducted from 0-7 days/week. Training can continue for a period from one to several months, depending on the particular task required, the previous experience and inherent trainability of the animal, and the skill of the trainer.

See 'Procedures Schematic' attachment for the timeline of the manipulations.

Procedures done on a single animal:

Please indicate how many and which procedures a single animal will go through. If applicable, please identify the strain/genotype/breed of animals that will be used in each procedure. Charts are highly recommended for clarity.

The maximum number of procedures a single animal may undergo is 15. Examples are provided in the "Procedures Schematic" attachment.

Multiple Major Survival Surgery Description:

Describe why it is necessary to perform multiple major surgical procedures on the same animal. Indicate the length of time between surgeries.

Multiple survival surgeries are necessary and unavoidable for these experiments. Our experiments require a positioner to orient the animal to the task and prevent head movements during neurophysiological recording. All of our experiments require other surgical procedures to place the chronic, semichronic or acutre recording arrays. These implantations could theoretically be performed at the same time. However, both are lengthy procedures. In consultation with OLAC veterinarians, we determined that recovery is easier and safer for each animal if these are performed as separate procedures. An additional advantage of separation is that several months might elapse between each procedure, allowing better healing.

The interval between the implantation of the head positioner and the recording chamber or recording array implant is very variable since it depends on the type of behavioral task the subject is performing, which in turn constrains where within the subject's training we need to implant the head positioner. Consequently, it can be many months (up to 18) until the subject has learned the full behavioral task and is ready for implantation of recording chambers or chronic microwire array implant. The interval between placing the chambers and opening the calvarium depends on the speed of the subject's recovery. We wait until the subject is again working on the behavioral task. This typically takes about 7 to 10 days. The interval between opening the calvarium and repositioning the chamber is very variable and depends on the reasons for repositioning the chamber. For example, if the chambers are being repositioned because of inaccuracy in their original placement this would occur approximately 2 weeks after the previous surgery to open the calvarium. However, if additional behavioral training is required then the interval can be much larger.

Protocol Title: [redacted])

Approval Period: 09/19/2019-05/31/2021

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* * * Husbandry * * *

Animal Transportation None

If animals will be transported between facilities, laboratories or institutions (e.g., hand carried, vehicular, etc.), describe the methods and containment measures to be utilized. Transportation of animals must conform to the ACUC Animal Transportation Guidelines.

OLAC has already established procedures for transporting animals from the vendor.

For behavioral testing, monkeys are moved in commercial primate chairs from their housing room to the testing room covered with opaque fabric.

For MRI scanning, monkeys are sedated and moved to the scanner room in a cart by the Vet and AHT staff.

FIELD STUDIES: If animals (live or dead) will be transported to or from the field, describe how they will be transported and measures to be taken to avoid potential disease transmission to researchers and other animals. Transportation of animals must conform to the ACUC Animal Transportation Guidelines.

N/A

Non-Standard Housing Requirements X None

Please check and describe all non-standard housing requirements that apply. Provide justification for each. For guidance, please refer to ACUC's Guidance on Exceptions Regarding Housing or Husbandry of Laboratory Animals, Aquatic Frog Housing Density, Guidelines for Investigators Who Manage Mouse Breeding Programs, and Rat Housing Guidelines.

Non-Standard Housing Requirements

Species	Cage/Pen Size	Cage sanitation interval	Wire-bottom rodent cages or grids	Animals outside dedicated animal housing for greater than 12 hours	Exemption from exercise (dogs only)
Monkey, Rhesus (OLAC Vivarium)					

Description of Non-Standard Housing Requirements

Non-Standard Husbandry or Care X None

Please check and describe any non-standard environmental requirements, diets, husbandry equipment or animal care. Include which species are affected. For guidance, please refer to ACUC's Guidance on Exceptions Regarding Housing or Husbandry of Laboratory Animals and Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Investigator Care of Animals (describe below and provide justification). For guidance, please refer to the ACUC Guidelines on Investigator Care of Vertebrate Animals.

Non-Standard Lighting Cycles, e.g., greater than twelve-hour light or dark cycles (describe below and provide justification).

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Non-Standard Housing Temperature Ranges (describe below and provide justification).

Non-Standard Diets (describe below and provide justification). For guidance, please refer to the ACUC Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Telemetry or Tether Devices (describe below and provide justification).

Running Wheels (describe below and provide justification).

Individually Housed (describe below and provide justification).

Exemption from Standard Enrichment (describe below and provide justification). For guidance, please refer to the ACUC Environmental Enrichment Guidelines.

Other - Please describe and provide justification.

Non-standard Experimental Requirements

Food or Fluid restriction None

Complete all section below that apply. For guidance, please refer to the ACUC Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Food or Fluid restriction

Species	Food Restriction	Length of Restriction	Fluid Restriction	Length of Restriction	Reason for Restriction
Monkey, Rhesus (OLAC Vivarium)			X	up to 22 hrs per day	See attached

Restraint of Conscious Animals None

Complete all section below that apply. For guidance, please refer to the ACUC guidelines on Physical Restraint of Unanesthetized Animals.

Restraint of Conscious Animals

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Species	Type restraint (manual, commercial, manual and commercial)	Describe acclimation to restraint	Length of restraint
Monkey, Rhesus (OLAC Vivarium)	Commercial	See attached	4-6hrs

Description of Restraint

During training and recording, animals sit in a commercial, specially designed chair that permits both limb movements and postural adjustments. Vendors include bKin, Primate Products and Crist Instruments. They adapt readily to chair training procedures. Animals who are required to learn a complex behavioral task may receive a substantial period of training in the chair before surgery. To condition animals to any head restraint that may be used, session duration is gradually increased from very short to normal length sessions. Head restraint, if used, will consist of the chair fixation post attached either to the animal's headpost. During behavioral training, it is rarely necessary for head restraint to be greater than 3-4 hours per day. During electrophysiological recording, however, it is often necessary to extend the period of head restraint up to (but not beyond) 8 hours per day. The majority of recording sessions take less than 6 hours, but the additional 2 hours will provide us with room to troubleshoot technical issues should they arise without having to abort the session. Because of the length of head restraint, the chair is adjusted each day for maximum comfort, and the animal is free to move its limbs and adjust its posture in the chair. In addition, tasks have been designed to minimize stress on the animal by giving it as much control over its behavior as possible. The behavioral tasks consist of many short (typically <5-s) trials each of which, if performed successfully, rewards the animal with fluid. When an animal gets tired or has had enough fluid, it simply stops performing the task. Animals typically become quite used to the laboratory environment and often fall asleep if their task is not available (i.e. if the program is not running) or after they have had all the fluid they are willing to work for in a given day. Daily length of chairing times are recorded along with the total fluid intake of the animal. We have a protocol in place to allow us to leave animals unattended for up to 20 minutes. This allows staff to take brief breaks for the bathroom and to eat and drink. A notice is displayed that alerts personnel that an animal is being tested and the staff member records the time at which they leave the animal and the time at which they return, ensuring that no animal is left for longer than 20 minutes.

Protocol Title: [redacted])

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Animal Disposition * * *

Please consult the ACUC Euthanasia Guidelines. Physical methods of euthanasia must be performed under anesthesia. Following euthanasia and prior to carcass disposal, an additional physical means of ensuring euthanasia must be performed. These physical methods vary by species but may include cervical dislocation for small rodents, bilateral thoracotomy, decapitation, exsanguination, double pithing for amphibians and reptiles, freezing for small ectotherms, or another AVMA-approved method. These must occur after the animal has been rendered non-responsive to noxious stimuli by the primary euthanasia agent.

Euthanasia

Protocol Title: [redacted])

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Species	Method of Euthanasia: Primary	Route of Administration	Dosage (in mg/kg if possible) and volume	Site	Building Name	Room Number	Method of Euthanasia: Secondary	Briefly describe the euthanasia procedure
Monkey, Rhesus (OLAC Vivarium)	Pentobarbital Overdose	Intravenous (IV)	At a minimum 1 mL per 10 pounds of body weight (after isoflurane overdose).					If used, the euthanasia procedure will take the following form. Animals will be euthanized with a large overdose of Pentobarbital. This is achieved with a pentobarbital-based euthanasia solution (390 mg pentobarbital sodium and 50 mg phenytoin sodium per mL), after isoflurane anesthetic overdose, verified by checking for absence

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

								of heartbeat. Euthanized animals are perfused and their brains removed for later analysis. All tissue not critical for experimental verification and analysis will be made available to other laboratories.

Provide specific details for carcass disposal.

Remains are placed in red bags/barrels and placed in appropriate cold room for carcass storage until proper disposal as biohazardous waste.

Protocol Title: [redacted]

Approval Period: 09/19/2019-05/31/2021

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* * * Attachments * * *

NOTE: The following types of files can be attached here: pdf, gif, jpeg,jpg, docx, xlsx.

Other Documents

Document Type	Document Name	Attached Date	Submitted Date
Other Documents	acclimation to restraint	03/23/2015	12/16/2015
Other Documents	water regulation	03/23/2015	12/16/2015
Other Documents	procedures schematic	08/06/2015	12/16/2015
Other Documents	APV Cranial Implant Care Guidelines	09/15/2016	09/15/2016

SOPs

Document Type	Document Name	Attached Date	Submitted Date
SOPs	fluid capillary loading and cleaning SOP	07/16/2015	12/16/2015
SOPs	Away from animal SOP	05/23/2016	07/20/2016
SOPs	[redacted]_R299_MRI NHP SOP_9.28.18	10/02/2018	10/02/2018

Protocol Title: [redacted])

Approval Period: 09/19/2019-05/31/2021

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* * * Certifications * * *

Certification

As Principal Investigator, I have ultimate responsibility for this study, the protection of animal subjects, and strict adherence by all co-investigators and research personnel to federal regulations, state statutes, and University of California (UC) Office of the President (UCOP) and UC Berkeley (UCB) policies pertaining to animal use in research and teaching.

I hereby assure the following:

1) As per the ACUC's Policy and Procedures on Protocol Review, any changes in the care and use of animals involved in this protocol will be promptly forwarded to the ACUC for review. Such changes will not be implemented until approval is obtained from the ACUC. I understand that the ACUC and Institutional Official (IO) have the authority to suspend a previously approved protocol if an activity is performed differently from that outlined in the protocol.

2) All procedures involving animal subjects will be performed under my supervision or that of another qualified professional listed on this protocol. Individuals listed on this protocol are qualified or will be trained to conduct procedures involving animals outlined under this proposal as per the ACUC's Training and Education Policy.

3) As per the ACUC's Training and Education Policy, all individuals listed on an this protocol have completed the required Collaborative Institutional Training Initiative (CITI) course, "Investigators, Staff, and Students - Basic Course".

4) As per the ACUC's Animal Occupational Health and Safety Program (AOHSP), all individuals working on this protocol have enrolled in the AOHSP by submitting an Occupational Health Surveillance System (OHSS). I understand that further participation in the AOHSP is voluntary unless required by the Occupational Health Physician or if the individual is working with specific species or research material.

5) The research proposed herein is not unnecessarily duplicative of previous reported research.

6) I ascribe to all of the responsibilities outlined in the ACUC's Principal Investigator Responsibilities policy.

X As Principal Investigator, I have read and agree to abide by the above obligations.

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Document Name	Created Date
acclimation to restraint.docx	09/17/2019

Training Summary

Phase 1

Pole and collar training and chair training using food rewards. Free waterline is attached. Fresh fruit is only given during training.

Habituation to:

1) The handler
2) Coming to the front of the cage
3) Pole on collar
4) Coming out of the cage
5) Climbing into the chair
6) Having the neckplate secured in the chair
7) Being covered and rolling in the chair

Phase 2

Habituation to experimental setup using food and fluid rewards. Free waterline is attached. Fresh fruit is given only during training.

Habituation to:

1) Laboratory
2) Positioner (if present)
3) Juice dispenser
4) Levers

Phase 3 (procedures: neural recordings and stimulation during cognitive testing)

Training on progressively more difficult behavioral tasks. Animal may drink to satiety during session. Free waterline is disconnected. Fresh fruit is given Friday and Saturday. Dry treats are given on weekdays.

1) Respond to visual cues on screen
2) Move levers
3) Fixate cues

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Document Name	Created Date
water regulation.docx	09/17/2019

WATER REGULATION

During behavioral training and recording, animals are kept on a water schedule. Access to water is regulated except during the daily training or recording sessions (up to 6 hours/day). During this period liquid rewards are used as positive reinforcement in shaping the animal to perform the required task, using operant conditioning techniques. Extreme care is taken to ensure that these procedures do not stress the animals.

Naïve animals can be chair-trained using food or juice rewards alone, without water restriction. Once habituated to the chair and experimental setup with operant conditioning, the free-water line will be removed from the home cage. This means that the animal will already be very familiar with the delivery of fluid through the juice dispenser. The animal will then determine its fluid allotment daily by performing the behavioral task for fluid rewards until satiated. Animals will be returned to their cage when they no longer perform the task for fluid. On weekend days if the animals are not run, they will receive water in their cages, equivalent to their average consumption. If the animal does not earn its minimum daily allotment in the session, the difference will be supplemented in a water bottle on the home cage.

Whenever an animal is on a water schedule, detailed records of fluid intake are maintained. During this period animals are also weighed at least weekly, and usually several times per week. When animals are not on a water schedule but they have been chair trained they will get weighted at least once a month. Skin and stool condition and general health are also monitored closely. Records are maintained in the animals' quarters and in the laboratory where they are available to veterinary staff and inspectors. Pair-housed animals that are not on water schedule, but which are housed with a partner that is on such a schedule, are offered water freely during the partner's laboratory session. Each monkey's "normal" body weight is determined at least once annually, by taking its weight after a period of at least one week of ad libitum food and water access.

The fluid intake of animals on a water schedule is monitored extremely closely to ensure that the animal gets sufficient daily fluid. Supplementary hydration is provided if the animal's weight falls below 90% of normal (determined as stated above), if it appears listless or unduly distressed, if the skin is loose and inelastic, or if stools are hard and dry or if the animal is constipated. Normally rehydration is achieved by suspending training and/or recording for several days so that the animal can be placed on ad libitum water.

Recording is typically carried out 3-7 days per week. Typically, training will take place 5 days per week. When an animal has a single day off or is moving from a water schedule back to ad libitum water, care is taken to prevent behavioral stress. When experiments are not being performed during weekends, the animal may be given an amount at least equivalent to their average consumption.

UCB Investigator Guidelines for Special Food/Water must be followed for water-regulated animals.

At least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin until post-surgical analgesics are no longer being given.

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Document Name	Created Date
fluid capillary loading and cleaning SOP.docx	09/17/2019

Fluid Capillary Loading and Cleaning Procedures

Materials:

Three 28 gauge hypodermic needles (one each for drug injection, air cleaning and fluid cleaning).

Three 10 - 30cm PORTEX Polythene inlet tubes with 0.28mm inner diameter (one for use with each of the three 28 gauge hypodermic needles).

One 5µL Hamilton syringe for drug delivery (Type 75, N. tip 2).

One Hamilton DS Digital Dispenser or homemade dispenser that fits the Hamilton syringe.

One 1mL syringe for filling.

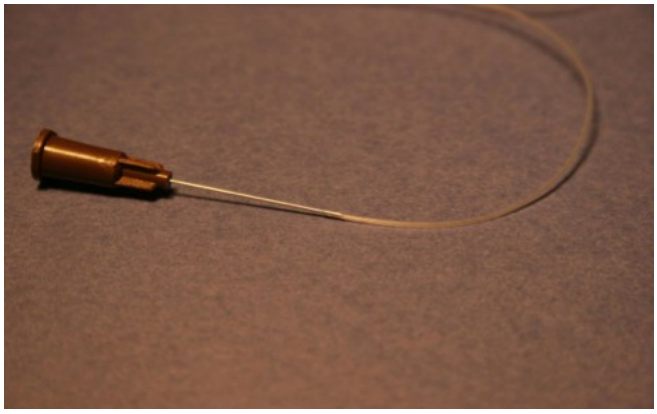
One 5 - 10mL syringe for cleaning.

Distilled and filtered water.

Cotton tip applicator.

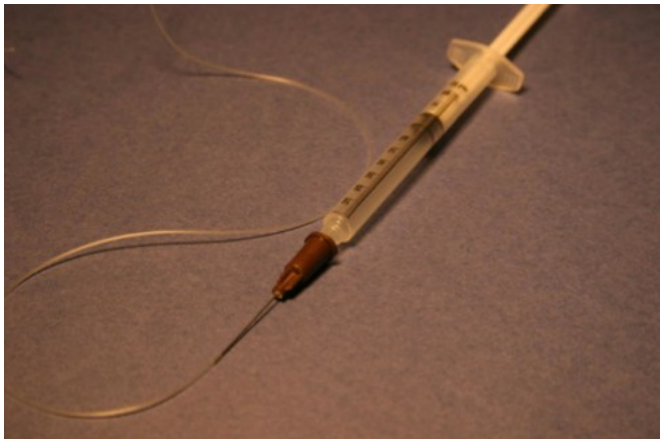
Loading Instructions (Steps 1 - 5):

Step 1: First prepare three sets of inlet tubes (one for the drug delivery, one for air cleaning and one for fluid cleaning) by attaching one inlet tube to each of the hypodermic needles (be sure you do not puncture the tube and it does not leak at the junction) — see picture below. The fluid going into the probe has to be distilled water or isopropyl alcohol based solution without solids (i.e. water soluble or isopropyl-soluble).



Step 2: Using one of the three prepared inlet tubes and the 1mL syringe, fill the syringe with drug solution (make certain air does not get inside). Use the hypodermic needle to guide some drops into the inlet tube of the needle from the 1mL syringe. Aim it to the side of the inlet tube to assist in preventing air bubbles from entering. If the inlet tube is filled, attach the 1mL syringe so that no air enters. Gently push the syringe so you can see that the fluid goes slowly into the inlet tube. Use plenty of light to see the front end of the fluid. If air is trapped inside, let in enough fluid to push out the bubble. If no bubble exists in the tube, take it down and fill up the Hamilton syringe.

NOTE: To prevent air from entering the Hamilton syringe, it is recommended to practice before performing the actual experiment by drawing solution up slowly, then quickly pushing back the knob of the syringe with the index finger. Push fast, but not too long. This will dispel the air. Then repeat the process until all air is eliminated. Keep the needle of the Hamilton syringe in the

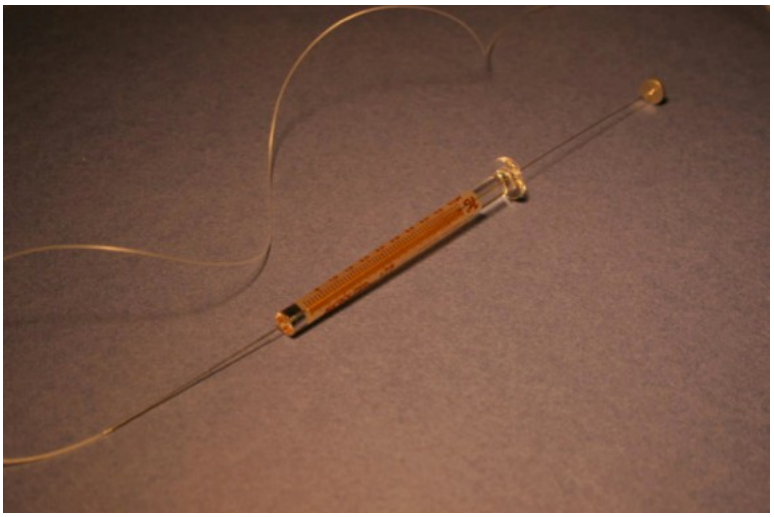


solution at all times during filling.

Step 3: After the Hamilton syringe is filled up, put it into the dispenser. Release some of the drug solution until a drop forms at the end of the needle. Take the filled tube/needle/1mL syringe complex and attach it to the inlet of the electrode. Gently push the tube onto the needle, and ensure it is not leaking. At this time, inject the drug solution through the system. Use a microscope to verify the drug solution is coming out the other side. Dispel enough drug solution to eliminate any bubbles.

Step 4: Attach the Hamilton syringe to the tube by first removing the needle from the tube. Push gently on the 1mL syringe and carefully remove the tube from the needle. If air remains, simply pick up the syringe with the dispenser, force out a drop of drug solution and attach the free end of the tube to the syringe. Set up is complete.

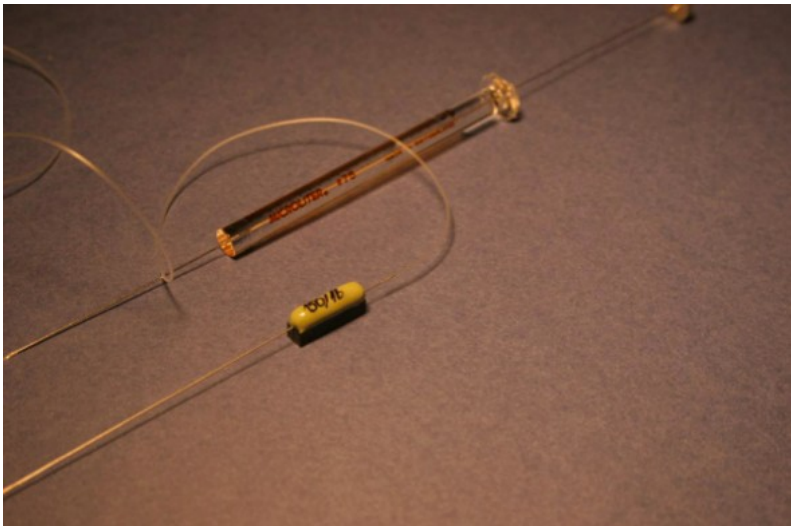
Step 5: Inject some drug solution to verify it works properly. Before implanting the probe, dip the V-Probe with the capillary into distilled water and blot up with a wet cotton tip applicator to minimize contamination.



Cleaning Instructions (Steps 6 - 11):

Step 6: Upon completion of the recording(s) and injection(s) with the V-Probe, gently pull the probe out of the brain and dispense the remaining drug solution from the Hamilton syringe.

Step 7: Fill the second prepared tube/needle and 5 - 10mL syringe with a combination of distilled and filtered water for use with cleaning.



Step 8: Remove the inlet tube from the electrode and attach the cleaning tube to it.

Step 9: Run a generous amount of water through the tube, and be certain the water filters out of the tube.

Step 10: Remove the tube from the electrode and force air from the third prepared tube/needle set until only air remains.

Step 11: The assembly is now ready to be gas sterilized before future use.

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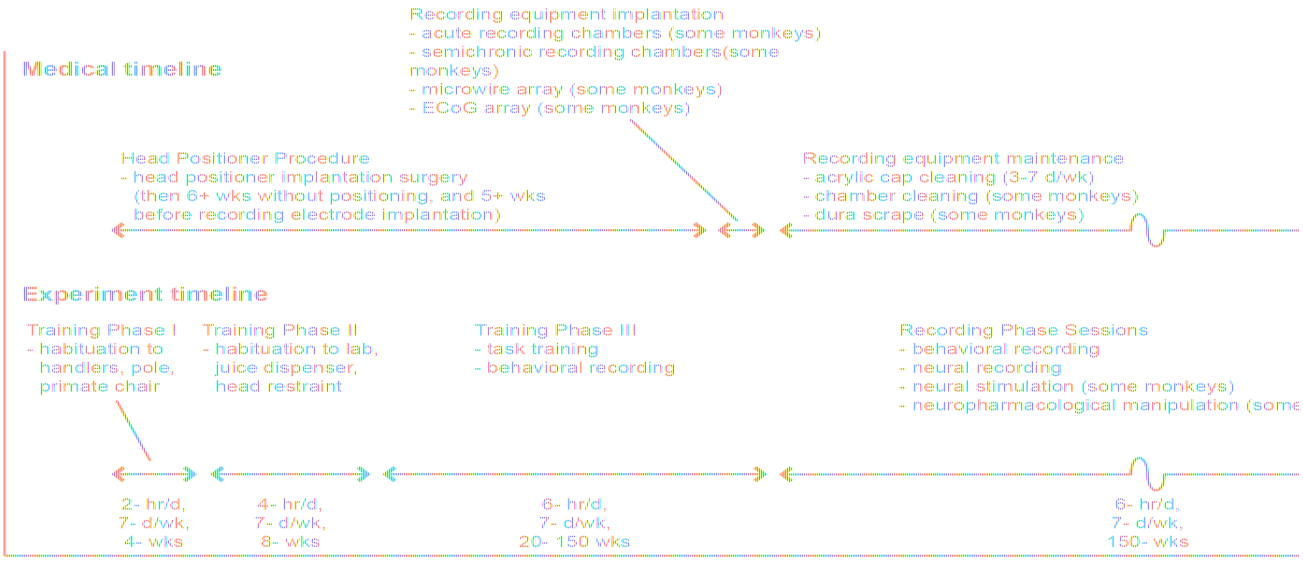
Document Name	Created Date
procedures schematic.docx	09/17/2019

PROCEDURES SCHEMATIC



An MRI scan will be performed for some monkeys at the beginning of training. A further scan may be necessary to verify that the electrodes are in the correct position following implantation. Additional scans may be required, for example, if scanning quality is poor or if the position of the recording chambers in the case of semichronic arrays needs to be adjusted and re-verified.

Alternatively, animals may be implanted with a semichronic or acute array instead of the chronic arrays. In such case, 3 surgical procedures to place a chamber, open the calvarium and insert the microdrive respectively will be performed.

In addition, an MR scan for chamber location verification could take place in between ‘Calvarium Opening for Semichronic Arrays’ and ‘Neural Recordings and Microstimulation using Semichronic Arrays’.

The following procedure lists are supposed to represent typical, as well as worst case scenarios. They enable us to deal with a variety of different causes of uncertainty. For example, although we are improving our protocols for MRI scanning, there are still occasions when the scans are not of sufficient quality. As another example, there are a variety of reasons that chambers might not be in an optimal position, such as difficulties in positioning encountered during surgery. Finally, we don't really understand how the brain works, so while we make reasoned estimates as to the brain areas or brain signals that we need to record, we can simply be wrong, requiring a modification of our measurements (for example, repositioning the chamber to target an alternative brain structure or switching from recording single units to local field potentials). The total number of procedures will not exceed 15.

Some examples of how this might work in practice are shown below. There will always be a minimum interval between two surgical procedures of 1 week. The maximum interval between two procedures is 2 years reflecting the length of time that it can take to get the animal’s behavior to the required state.

MRI scan → Positioner implantation → Chamber placement for acute array → Calvarium opening for acute arrays → Neural recordings using acute arrays → Dura maintenance → Dura maintenance (if first procedure was insufficient) → Intracranial injection (Muscimol inactivation to test causality of relationship between observed neural signals and behavior)

MRI scan → Positioner implantation → Chamber placement for semichronic array → Calvarium opening for semichronic array → Microdrive insertion → Neural recordings using semichronic arrays → MRI scan (to detect electrode positions accurately) → Removal of semichronic array → Dura maintenance → Microdrive insertion → Neural recordings using semichronic arrays → MRI scan (to detect electrode positions accurately)

MRI scan → MRI scan (1st scan poor quality) → Positioner implantation → Chamber placement for semichronic array → Calvarium opening for semichronic array → Microdrive insertion → Neural recordings using semichronic arrays → Neural stimulation → MRI scan (to detect electrode positions accurately) → Removal of semichronic array → Dura maintenance → Microdrive insertion → Neural recordings using semichronic arrays → Removal of semichronic array → Intracranial injection (Muscimol inactivation to test causality of relationship between observed neural signals and behavior)

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Document Name	Created Date
Away from animal SOP.docx	09/17/2019

Standard Operating Protocols: Away from Animal SOP

1. Should be wearing all PPE prior to handling NHP:

1. lab coat
2. face shield & face mask
3. gloves
4. scrub pants
5. hair net - shoe covers

2. Check to see if NHP is working normally by noting that the animal's eye is visible in the eye tracking camera

3. If eye is not visible, open behavior box to do a visual check to ensure that the NHP is in normal condition

4. Fill out Away from Animal Log with required information:

Date, NHP name, contact number, leave time, and initials

5. Place sign on door indicating animal is unattended in the room

6. Leave room for no longer than 20 minutes ensuring doors are closed in the room containing the NHP

7. After arriving back to NHP room, open metal window door to see if the NHP is loose in the room

8. If the NHP is not loose, enter room, ensuring door is closed behind you*

9. Determine if the animal is working normally by once again noting stable eye movement by looking at the eye tracking camera

10. If eye is not visible, open behavior box to do a visual check to ensure that the NHP is in normal condition

11. Fill out remaining fields in the Away from Animal Log:

Arrive time, total time

12. Remove sign from door

13. Resume experiment

***If the NHP is loose, call 643-VETS (510-643-8387)**

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Document Name	Created Date
APV Cranial Implant Care Guidelines.pdf	09/17/2019

Association of Primate Veterinarians
Cranial Implant Care Guidelines for Nonhuman Primates in Biomedical Research

PURPOSE

Use of nonhuman primates (NHPs) in biomedical research may include performing invasive cranial surgeries with chronic implantation of research devices. The Association of Primate Veterinarians (APV) supports the responsible use of NHPs in neurobiological research. Such research must meet specific criteria, such as the institutional animal care and use committee (IACUC) review and approval, verification of the investigator's skill and experience, and establishment of a close working relationship with institutional veterinary staff (Guide 2011). The following text aims to provide nonhuman primate researchers, IACUCs, and veterinary staff with guidelines for conducting research involving chronic cranial implants and for assessing their routine and non-routine care.

BACKGROUND

Success in maintaining a chronic cranial implant in operational condition is a function of how the implant is placed and the types of materials used, coupled with the animal's physiology and healing responses. The laboratory animal veterinarian should interface closely with the research group to ensure the adequacy of training and use of optimal surgical technique. Cranial implantation surgery must be conducted with consideration of normal host anatomy and physiology, as well as the maintenance of aseptic technique. With some surgical implant procedures, it is common to stage placement of cranial implants. Waiting to place each attachment (e.g. recording chamber) until the data recording is required helps to preserve the integrity of the chamber and safeguard the health of the animal. The total number of allowable cranial surgeries should be reviewed and approved by the IACUC.

GUIDELINES

Pre-surgical procedures

Clipping the hair liberally around the surgical site while avoiding clipper burns and cuts will help minimize unwanted irritation and infections. Small scissors or commercial depilatory products can be used in areas inaccessible for clipping. The skin must be surgically prepared, disinfected, and draped in a sterile fashion.

Surgical procedures

1. Cranial implantation surgeries must employ techniques that minimize trauma and preserve tissue architecture. A combination of aseptic technique, appropriate instrument and suture use, isotonic fluid lavage, and skillful and gentle tissue handling is highly recommended.

2. A neat and sterile cranial surgical site provides the best bonding surface, promotes bone remodelling, and facilitates anchoring of the cranial implant to the skull.

3. Use of a high-powered drill by an inexperienced surgeon may lead to thermal cranial damage and secondary local bone necrosis with loosening of the screws and eventual implant detachment. Hand drills do not cause thermal damage, but their use can lead to larger than necessary holes due to their poorer stability. Continuous lavage with cold isotonic fluids during drilling or application of thin layers of exothermic compounds (e.g., methacrylate) may help prevent or minimize thermal damage to the bone and periosteum. This kind of damage may be particularly important in younger or smaller NHPs with a thinner cranium.

4. Titanium or high quality stainless steel orthopedic screws are often used to anchor cranial implants. Drilling pilot holes combined with the use of bone taps and blunt tipped screws will minimize or even eliminate bone damage while contributing to implant longevity (Abee 2012).

5. Hemostatic materials such as Gelfoam®, are effective in stopping acute bleeding but they must not be left inside the cylinder indefinitely. To remove Gelfoam® the cylinder should be filled with sterile saline for approximately 10 min to soften residual foam pieces for removal and the process repeated, if needed. Forceful removal of Gelfoam® residue may produce additional hemorrhage and should be avoided. Cranial bone edges are the most common source of bleeding within the cylinder and this can be controlled by sealing the edges with bone wax. The implanted cylinder may be opened after the surgery for visual examination and carefully cleaned 1-2 days after surgery. After assuring adequate hemostasis, 2-3 ml of sterile saline should be placed in the cylinder followed by aseptic replacement of the cap for another 2 to 3 days. Routine cleaning and maintenance of a non-infected cylinder should be initiated in 1 week post-op.

Post-surgical procedures

While tending to the newly placed or chronic cranial implants one should be vigilant about potential pain. If there is any evidence of pain or distress associated with routine cleaning the underlying cause should be investigated, addressed, and appropriate analgesia given. One or a combination of the following agents is recommended: EMLA cream, lidocaine jelly, lidocaine or bupivacaine local block, or systemic NSAIDs or opioids.

1. <u>Wound Margin Care</u>

    a. An uninfected surgical wound that is healing well is best left alone for a period of 7-14 days post-operatively. Sterile saline rinses can be used if needed to clean the wound. Use of H_2O_2 is not recommended for 2-3 weeks post-op as it can interfere with normal healing process. Dry, non-infected, hard and crusty scabs formed during normal healing may cause local irritation or pruritus, inviting self-trauma. Petroleum jelly or wet dressings applied every 2-3 days will keep the scabs soft and facilitate healing. There is no universally recommended frequency of cleaning. Rigorous, over exuberant, unwarranted cleaning can result in inflammation and infection. Wound margins should be closely inspected a minimum of once each week and cleaned as often as needed.

b. Re-growing hair should be carefully removed on an as-needed basis.

c. The wound margin adjacent to an implant requires regular observation and attention as it may become infected leading to suture dehiscence or necrosis, resulting in areas of skin devitalization or retraction away from the implant. Daily cleaning may be necessary as the serous, serosanguinous or purulent secretions will dry up at the wound margin producing a protein-rich crust that may serve as a nidus of infection. Cleaning of the skin/implant interface involves gentle removal of loose crusts and of unwanted hair with a scissors and rinsing wound margins. The following solutions or their combinations should be considered for cleansing: sterile saline, chlorhexidine diacetate 0.05% solution (1:40 dilution of stock chlorhexidine with water) (Slatter 2003), povidone-iodine 1-2% solution (1:10 – 1:5 dilution of stock povidone solution to saline), Dakin's solution (0.5% sodium hypochlorite in water) can be used particularly in the presence of necrotic tissue or 1.5 - 3% hydrogen peroxide (to remove dried up blood and other secretions followed by copious saline irrigation). **None of the above compounds is effective indefinitely or against all pathogens and a 7 to 10 day rotation of different disinfectants should be employed.**

d. Enzymatic debriding compounds facilitate the process by which devitalized tissue is softened or liquefied and removed (e.g. Trypzyme ®, an enzymatic soaking solution).

e. Infected sites should be cleaned frequently (e.g. daily). Where mild but chronic skin/implant problems are evident, a minimum twice a week inspection and cleaning 3-4 days apart, are recommended. Culture and sensitivity should be done to ascertain the nature of the infectious agent. The indiscriminate use of systemic or local antibiotics may contribute to the development of bacterial resistance and is strongly discouraged.

2. Cranial Head-post Care

The skin may retract away from the head-post over a period of weeks to months post-operatively and this is usually a gradual process. In the absence of local infection, skin repair surgery may be attempted. If the skin retraction is significant, an addition of bone cement may be considered.

3. Routine Recording Cylinder Care

Most recording cylinders are anchored with screws and methacrylate products and have a tight fitting cap secured with 1-3 small screws. The inside of a chronic recording cylinder is not sterile but it must be maintained aseptically. Recording cylinders are routinely opened in the non-sterile environment of the research laboratory or procedure room. Careful cleaning of the recording cylinder as described below has been demonstrated to minimize or prevent active cylinder infections. Ideally, no smell should be detectable in the recording cylinder and the underlying dura should appear creamy white, smooth, and shiny.

a. The outside of the cylinder is typically contaminated and must be cleaned before the cylinder is opened for cleaning and/or recording. Povidone-iodine scrub (soap) should be

used for the initial scrub and washed off with saline or 70% alcohol. Residual blood may be removed with 0.75% to 3% hydrogen peroxide (H_2O_2). Care must be taken to avoid contact between alcohol or H_2O_2 and viable soft tissues that are in the process of re-epithelialization.

b. Sterile draping and aseptic techniques while opening a recording cylinder are recommended.

c. Uninfected cylinders should be cleaned as often as possible, but no less than twice a week 3-4 days apart. Sterile instruments (e.g., aspirator/suction tips, forceps) and supplies (e.g., gauze, drapes, gloves) should be used while working inside the recording cylinder. After cleaning, it is recommended that the old cap be replaced with a new sterilized (i.e. autoclave, Cidex®) cap each time. Although it may be less effective, but cleaning of the used cap with povidone-iodine scrub, alcohol, and rinsing or soaking it in a 1:10 sodium hypochlorite solution is used by some programs.

d. Known or suspect infected cylinders should be cleaned 5 to 7 days a week (Gografe & Niekrasz 2009), regardless of whether animals are treated with antimicrobial agents. If there are multiple cylinders, they should each be thoroughly cleaned sequentially rather than simultaneously. No materials (e.g. forceps, suction tips, etc.) should be shared between cylinders during multiple cylinder care. Uninfected cylinders should always be cleaned before suspect or known infected cylinders. Cleaning should always begin with a sterile saline lavage followed by suction. The dura must be carefully examined for the presence of focal infection, necrosis, cuts or tears before any cleaning agents are applied. Disinfectants and antibiotics (e.g. cephalosporins) may contribute to unwanted toxic events that manifest clinically as neurological deficits. The following compounds have proven useful:

    i. Use of a 3% H_2O_2 solution or a 1:1 mixture of H_2O_2 and povidone-iodine facilitates removal of biofilm and proteinaceous material from the interior surface of the cylinder wall.

    ii. Rinsing several times with a dilute povidone-iodine solution at 1-2 % (dilution is necessary for ionization of bound iodine). After cleaning, a few drops of 2% povidone-iodine solution may be left inside the cylinder.

    iii. Although some programs have reported the use of chlorhexidine inside the recording cylinder for routine maintenance without problems, its use is controversial, as the compound has been demonstrated to have neurotoxic properties (Henschen & Olson 1984, Perez, et. al. 2000, Lai, et. al. 2011). The US Physician Desk Reference (PDR), as of 1984, warns that "*chlorhexidine gluconate is for external use only. Keep out of eyes and ears and avoid contact with meninges*". Since other disinfectants (i.e. chlorine, iodine, etc.) have been demonstrated to be efficacious for cylinder maintenance, the use of chlorhexidine should be carefully evaluated. At a minimum, care should be taken to evaluate the

dural integrity prior to using chlorhexidine and to thoroughly rinse the cylinder free of the compound after each use. Leaving residual chlorhexidine in the cylinder for extended periods of time is also not recommended.

iv. Dakin's solution may be used cautiously when addressing infections refractory to other treatments and when the integrity of the dura has not been compromised.

v. Chlorine dioxide is typically not used in routine cleaning but it may be effective in short-term treatment of mycotic infections (Lee 1998).

vi. In the majority of cases involving a durotomy or durectomy, the underlying cortex is covered with artificial dura combined with the use of silicone membranes, collagen matrix, or aliphatic polyether polyurethane sheets. Where the dura has been cut, it should be sutured to protect the cortex. The cylinder cleaning process is the same as with intact dura. It is critical to rinse with copious volumes of sterile water or saline if any disinfectant was used.

4. Granulation tissue.

Granulation tissue (GT) formation is part of the normal healing process, but it is not always desired when maintaining chronic cranial implants. Budding GT on the wound margin and the dura is typically highly vascular and bleeds easily, oozes serum, and may interfere with healing if it becomes infected. Dural GT that is not removed on a regular basis may bleed and eventually result in dural fibrosis. Thick granulation tissue pads can harbor bacteria and become a source of chronic chamber infections.

a. 5-Fluorouracil (5-FU) may be helpful in reducing or delaying the GT growth (Spinks 2003). 5-FU is an antimetabolite, antimitotic agent that reduces tissue re-growth, vascularization, and bacterial overgrowth by interfering with nucleic acid synthesis, thus preventing mitosis. 0.5-1.0 ml of 25 mg/ml aqueous 5-FU should be instilled into the cylinder three times weekly to bathe the dura for 5 minutes. At the end of 5 minutes the cylinder should be rinsed with copious volumes of sterile saline. 5-FU must never be used on compromised dura as subdural leaks may contribute to complications. 5-FU decreases fibrinolytic activity and enhances the risk of thromboembolic events (Kessler 1994). Care must be used when handling 5-FU because it is a known carcinogen.

b. Early GT deposits may be removed using suction. Local anesthesia can be provided via instillation of 0.25-0.5 ml of 1-2% lidocaine or 0.25% bupivacaine or a 50:50 mixture for a few minutes before removal. Post-procedural systemic analgesics should be considered.

c. Chronic growth of GT typically leads to the formation of a firm fibrous layer requiring "dural scraping", which must be conducted under general anesthesia with the post-operative use of analgesics. GT deposits on the wound margin may be addressed by surgical debridement followed by a V-plasty, regular cleaning, treatment of local infections, and chemical or electrical cauterization under systemic or local anesthesia.

5. Treatment of Implant Margin and Cylinder Infections

While assessing the skin/implant interface, care must be taken to determine if the interface infection is topical or originating from under the cranial implant. In addition, the inability to retain fluid within the recording cylinder is often the result of open tracts between the cylinder and wound margin. The ideal interface should be smooth and free of "pockets" and abrupt changes in the contour of the implant. Reshaping of the interface and performing a "V-plasty" should be considered. Culture of purulent exudate, cleaning/debridement of the area, and administration of topical or systemic antibiotics have also been used.

Infections inside the recording cylinders are common and can be prevented and treated with careful cleaning and maintenance as outlined. Systemic antibiotics should be reserved for treating cylinder infections in which the dura or bone are severely compromised or where the infection has been unsuccessfully treated with the frequent cleanings and use of halogen solutions. Indiscriminate use of antibiotics can result in bacterial resistance and additional problems. The use of halogen solutions within the cylinder (e.g. Povidone-iodine, Dakin's solution, etc.) has been demonstrated to clear infections in many cases. These solutions have been used as part of the cleaning regimen and have been left in the chamber after cleaning for extended periods to treat chronic infections. Chronic infections should always be treated in consultation with a veterinarian.

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UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted] ([redacted])

Approval Period: 09/19/2019-05/31/2021

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

*** * * Attached Document * * ***

Document Name	Created Date
[redacted]_R299_MRI NHP SOP_9.28.18.docx	09/17/2019

MRI NHP SOP

Ahead of time, magnet will be booked for 2 hours.

Personnel

- All personnel allowed to enter Zones III (operator: [redacted]) and IV (magnet: [redacted] must have passed the BIC User Safety training (http://bic.berkeley.edu/scanning).
- All personnel must have undergone Herpes B training.
- Number of personnel need to be kept to a minimum to ensure safety around magnet. For each stage of the protocol, personnel should have definite roles.
- Number of personnel simultaneously in room with magnet should be kept to a minimum to avoid diffusion of responsibility.
- Protocol requires four personnel: vet, AHT, PI (or another NHP PI with a currently approved Animal Use Protocol, or another NHP trained lab member from a lab with a currently approved AUP), and one lab member. One member of the team will be a qualified scanner operator, or an additional qualified Research Associate (hired by the lab) will be used.

Equipment

- Fully enclosed transportation cart for animal
- Veterinary cart with supplementary supplies (needles, syringes, additional ketamine, atipamezole, towels, ET tube, laryngoscope, lidocaine, thermometer, portable pulse oximeter)
- Anesthesia machine with full oxygen tanks (including extra isoflurane, tubing with appropriate connectors, extra anesthetic masks)
- MRI compatible stereotactic frame
- MRI compatible monitoring equipment (pulse oximeter)
- Portable NHP exposure kit (to remain in [redacted]
- MRI cleaning cart
- All equipment designated for use within the magnet on the must be approved ahead of time by Ben Inglis and marked with MRI compatible sticker

Procedure overview

1. Induction

Procedure	Room	Responsibility
Anesthetic induction (2-3mg/kg ketamine, 0.015-0.04 mg/kg dexmedetomidine, 0.1-0.25mg/kg midazolam) and catheter placement for IV access	[redacted]	Vet, AHT
Shave area for placement of respiratory monitor	[redacted]	Vet, AHT
Remove primate collar	[redacted]	Vet, AHT
Endotracheal intubation (optional; this step performed if stereotaxic frame will be utilized)	[redacted]	Vet, AHT

	[redacted]	

2. MRI decontamination (beginning)

Procedure must be performed at least 20 minutes before the arrival of the animal to ensure sufficient air changes.

Procedure	Room	Responsibility
Transport equipment to scanner		PI , Lab member
Placement of barriers and signage	[redacted]	Lab member
Black out windows from operator room to hallway		Lab member
Lay down plastic sheeting in magnet room from doorway to scanner		PI
Tape down metal sheet in scanner doorway	[redacted]	Lab member
Remove wall panels close to doorway in magnet room		PI
Remove patient bed and insert tray for animal		PI
Surface decontamination (bed, coil and bore) using Clorox disinfectant wipes		PI
Lay down plastic sheeting in operator room from doorway to magnet room	[redacted]	Lab member
Prepare vital sign monitoring equipment	[redacted]	PI, Lab member

3. Transport of animal

Procedure	Room	Responsibility
Record baseline vitals (heart rate, respiratory rate, temperature, SpO2)	[redacted]	Vet, AHT
Call operator room in BIC before leaving ([redacted])		Vet, AHT
Transport animal to scanner in cart		Vet, AHT
Spray cart wheels with NPD on leaving vivarium		Vet, AHT
Remove shoe covers on leaving vivarium and place in biohazard bag		Vet, AHT

4. Positioning of animal in scanner

Procedure	Room	Responsibility
Animal lifted from cart to magnet	[redacted]	Vet, PI
Place animal~~monkey~~ in ventral recumbency in stereotactic device*		Vet, PI
Position pulse oximeter and respiratory monitor	[redacted]	Vet, AHT
Slide head into head coil		PI
Monitor vital signs at least every 10 minutes (heart rate, respiration, SpO2)		AHT
Wrap patient in towels to maintain body temperature	[redacted]	AHT
Insert anesthesia tubing through wall conduit once wall	[redacted]	Vet, AHT

panel has been removed and tape to side of bed to ensure proper placement is maintained		
Initiate supplemental O2 (± isoflurane) via mask or endotracheal tube at 1-3 L/min	[redacted]	AHT
Index head with bed referencing system	[redacted]	PI
Insert animal into magnet via motorized bed		PI
All personnel leave room, RF-screened door closed		

* alternately position animal using disposable foam blocks and register position using fiducial markers (vitamin E capsules) taped to ear canals and eye orbits

5. Scanning

Procedure	Room	Responsibility
Monitor of vital signs at least every 10 minutes (respiratory, heart rate, SpO2)	[redacted]	AHT
MRI operator registers monkey as a subject and initiates scan protocol	[redacted]	PI
If vital signs degrade or there is reason to check on the animal's health, scanning is suspended and one or more personnel enter the magnet as necessary*	[redacted]	Vet
Should vital signs indicate that supplemental anesthesia is required, isoflurane will be administered at a dose of 2-3% via mask	[redacted]	Vet, AHT
If there is reason to check on the animal's position, scanning is suspended and one or more personnel enter the magnet as necessary		PI

* one member of vet staff will remain gowned in the event that the patient needs to be accessed for monitoring

6.Removal of animal from scanner

Procedure	Room	Responsibility
Animal removed from magnet via motorized bed	[redacted]	PI
Head removed from head coil		PI
Vital sign sensors removed		Vet
Animal removed from stereotactic device		PI
Animal lifted from magnet to cart and transported to anteroom		Vet, PI

7. Transport of animal

Procedure		Room	Responsibility
Transport animal to holding room		[redacted]	Vet

8. MRI decontamination (end)

Procedure must be performed at least 20 minutes before end of scanning block to ensure sufficient air changes.

Procedure	Room	Responsibility
Surface decontamination (bed, coil and bore) using Clorox disinfectant wipes	[redacted]	PI
Remove animal tray and replace patient bed	[redacted]	PI
Collect plastic sheeting	[redacted]	PI
Collect plastic sheeting	[redacted]	PI
Remove metal sheet from scanner doorway	[redacted]	PI
Replace wall panels as needed	[redacted]	PI
Remove restricted access signs and window black out	[redacted]	PI
Transport equipment back to animal facility		PI

9. Recovery

Procedure	Room	Responsibility
Provide supplemental heat via warm water recirculating blanket or warmed towels if needed; remove catheter; monitor vitals to ensure return to normal (including pink and moist mucous membranes, CRT< 2 seconds, and rectal temperature of approximately 99-102.5 F)	[redacted]	Vet, AHT
Replace primate collar	[redacted]	Lab member
Reverse dexmedetomidine if necessary with 0.15mg/kg IV/IM atipamezole	[redacted]	Vet, AHT
Remove endotracheal tube if placed	[redacted]	Vet, AHT
Monitor animal recovery	[redacted]	Lab member

Emergency procedures

A building occupant is required by law to evacuate the building when a fire alarm sounds. Good judgment is required while working with non-human primates. The following guidelines will assist you to safely and humanely respond to an emergency alarm.

In the event of a major earthquake or fire

1. If possible, enter the magnet room and euthanize the animal prior to evacuating.
2. If at any time you deem that there is an immediate threat to your safety, evacuate immediately.

In the event of a magnet quench

The magnet could quench either due to an earthquake, because a large metal object hits the magnet, or because it is activated manually using a dedicated emergency button. The quench should be controlled (i.e. vent to the outside) and take about 30-40s. However, if the quench is uncontrolled (e.g. the vent fails in the earthquake), the magnet room could fill with freezing cold helium gas.

1. If you think the magnet is quenching, you should first open the magnet room and operator room doors and prop them open, to prevent a pressure build-up and to allow fresh air in. Then, if you can do so safely, go into the magnet room (ensuring that the magnet room door remains wide open) to remove the subject from the magnet as quickly as possible.
2. If there are indications that the quench is uncontrolled (white vapor, substantial temperature drop, animal vital signs absent, low oxygen alarm above the operator console) do not enter the room until instructed to do so by BIC personnel.

All other responses to an emergency alarm

1. As soon as you hear the alarm, send someone to speak with the Building Coordinator.
2. In the interim, begin procedures to take the animal out of the magnet and return him to the animal facility.
3. If, in the judgment of the Building Coordinator, there is an immediate threat to human safety you will be instructed to evacuate.
4. If at any time you deem that there is an immediate threat to your safety, evacuate immediately.