University of California, Los Angeles
Chancellor's Animal Research Committee (ARC)

Amendment Application

General Information	
Title:	[redacted]
Protocol #:	[redacted]
PI:	[redacted]
Status:	APPROVED_WITH_CODICIL
Approval Period:	9/17/2019-7/17/2020
Received Date:	9/4/2019
Type:	Amendment
Species:	8 Rhesus Monkey (Pain Category D)
Create Date:	9/4/2019 8:47:48 AM
Created By:	[redacted]
Owner:	[redacted]

Updated Sections
Amendment Summary
Pain L terature Search
Phys cal Restraint
PI Assurance

Personnel Certifications Due:

[redacted]
- MHQ (val d until 2/26/2020)

Notes:

- General Certif cation Test: Offered through CITI program (**http://www.citiprogram.org**). Please ensure your affiliat on is listed as UCLA and complete the Animal Research Basic Course.
- Medical History Questionnaire (MHQ): Offered by the Occupat onal Health Facil ty (**http://mhq.healthsciences.ucla.edu/**).
- Species Specific Training: Please visit the DLAM webs te: **https://portal.dlam2.ucla.edu/EducationTraining/Pages/default.aspx**.

For more questions regarding certifications/training, please visit:
http://ora.research.ucla.edu/rsawa/arc/pages/certification_info.aspx.

Codicil(s):

- The Committee understands that [redacted] will not be used for activities involving the use of non-human primates until this room has been inspected and approved by the ARC as a Research Area. Similarly, IBC approval for the use of this area must be obtained pr or to use
- The Committee understands that paraformaldehyde and atropine will not be used in these experiments until EH&S approval for the use of these agents has been granted.

Amendment Summary

Please provide the appropriate information regarding changes to this protocol. Then update the respective sections. If this amendment is requesting a change in personnel, please indicate the individuals you are adding or removing by listing their names in the textbox for question #3 below.

1. Check the following if you will be making any of the following changes:

In addition to checking these boxes, you must update the respective sections of this protocol.

- ☐ Protocol title
- ☐ Funding or funding agency
- ☐ Principal investigator
- ☐ Co-investigator
- ☐ Personnel
- ☐ Location

2. Check the following if you will be making any Significant changes:

In addition to checking these boxes, you must update the respective sections of this protocol.

- ☐ Animal species and/or strain
- ☐ Number of animals
- ☐ Pain category
- ☐ Method of euthanasia
- ☐ Experimental procedures

A. If you indicated that you will be changing the number of animals above, please provide a detailed explanation of your rationale for the number of additional animals requested. Please note that if this request for additional animals also entails a change in experimental procedures and/or pain category, please update these application sections and indicate these changes on this page.

B. If you indicated that you will be changing the experimental procedures above, please provide a detailed explanation of how this change in experimental procedures relates to the experiments in your currently approved protocol. In addition, please clarify what results you hope to yield from this changes in experimental procedures.

3. In order to assist reviewers, briefly describe in lay terms the changes you are making and complete the appropriate sections. If this amendment is to change funding only, please assure the committee that the research is identical to the previously approved submission. If this amendment is requesting a change in personnel, please indicate the individuals you are adding or removing by listing their names below.

In order to clarify our pole training techniques, I have edited the answer to Q2 in the Physical Restraint section. I have made two changes to the answer. I now state, "There are two approaches we use for this step. In one, the animal is hooked in the cage and, once it is used to being on the pole, it is taken out of the cage and lured onto a scale by means of food treats..."

At the end of that paragraph, I have added the following text, "The alternative approach is to use a special training chair. Using this approach, the animal is able to get into the chair without being poled and the initial hooking and acclimatization to the pole occur while the animal is in the chair. In this approach, hooking the pole to the collar while the animal is in the cage occurs later in the process."

This new text clarifies that we sometimes use a special training chair and that, in these cases, the hooking of the collar in the cage is not the first step.

I have also updated the Pain Literature Search.

Following from the ARC review, I have updated the edited text to now say, "The alternative approach is to use a special training chair with a vertical sliding door. The training chair is designed so that it directly abuts the cage and when the cage door is opened, the animal can only go into the chair. The animal is lured into the chair with food rewards and does so without being poled. Once in the chair, the sliding door can be closed and the chair moved away from the cage. Using this approach, the initial hooking and acclimatization to the pole occur while the animal is in the chair. Hooking the pole to the collar while the animal is in the cage occurs later in the process."

Research Summary

Your answers to the questions on this page determine the other sections needed to be filled out.

1. What is the Title of the Project?

[redacted]

2. Check all that apply:

- ☐ Tumor Formation (spontaneous or implanted)
- ☐ Chronic Disease (diabetes, EAE, status epilepticus, etc.)
- ☑ Tissue Collection (blood and all other tissues, including those collected after euthanasia)
- ☐ Antibody/Ascites Production
- ☑ Surgical Procedures (survival, non-survival)
- ☑ Non Surgical Procedures (injection of experimental drugs, behavioral studies)
- ☑ Gas Anesthetic Agent(s) (use of isoflurane, halothane, etc)
- ☑ Hazardous Agents (carcinogens, paraformaldehyde, rDNA, vectors, etc.)
- ☐ Radioisotopes or radioactive implants
- ☑ Prolonged Physical Restraint (physical restraint of unanesthetized animals for periods longer than 15 minutes)
- ☐ Genetically Modified Animals
- ☐ Tissue Sharing (use of tissues only)

3. Will the research be conducted exclusively on tissue received from another investigator?

No

If yes, do your funding sources require an ARC approved protocol?

No

4. Check all that apply:

- ☐ Experiments done entirely at another institution
  NOTE: For experiments conducted entirely at another institut on please subm t the most recent approval not ce and a copy of the most recently approved protocol from the other institution with your submission. Please also indicate the PHS Assurance number and AAALAC accreditation status.
- ☐ Experiments done entirely at VAGLAHS
- ☐ Program Project/Training Grant
  Administrative approval only – no animals associated with this protocol.
- ☐ Breeding Colony: #
  NOTE: If you will be breeding animals for this protocol and do not already have an approved breeding protocol on file w th the ARC, you must submit an Appl cat on to Establish and/or Maintain a Breeding Colony at this time. Check the box above but leave the "Breeding Colony Number" field above empty. The ARC Staff will update the Breeding Colony Number following the submission of a breeding colony application.

5. If you are seeking approval for a training grant, list all individual projects supported by the program project or training grant, including the principal investigators' names and their current ARC approval numbers. If no animal research is currently being supported by the overall grant, please assure the Committee that, should an investigator of a project covered by the overall grant initiate research involving animals, ARC approval will be obtained prior to the distribution of funds.

Personnel

There can be only one Principal Investigator per protocol. To edit a person's contact information or add a new person to our system, click on the People tab above.

Prior to the submission of an amendment to add personnel, please ensure that these individuals have completed all applicable animal use certification requirements and have a Medical History Questionnaire (MHQ) on file with the Occupation Health Facility (OHF). If you are only requesting the removal of personnel, please email the ARC administrative office (arc@research.ucla.edu). An amendment application is NOT required if you are only removing personnel.

Principal Investigator

[redacted] View Person Detail

Email:	[redacted]	UID:	[redacted]
Phone:	[redacted]	Degree:	[redacted]
Fax:	[redacted]	Dept:	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Principal Investigator

Which species will this person handle in this protocol?

Rhesus Monkey

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr. [redacted] has worked with non-human primates since [redacted] working with Macaca nemestrina, Macaca fascicularis and Macaca mulatta. [redacted] has undergone non-human primate handling and safety courses at the [redacted] in [redacted] the [redacted] the [redacted] and [redacted] and [redacted] is experienced with all of the procedures indicated in this application. [redacted] has published numerous articles in journals such as Science, Nature Neuroscience, PNAS, the Journal of Neuroscience, Cerebral Cortex, Experimental Brain Research, and the Journal of Neurophysiology using these types of experimental preparations.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

The PI will perform the following duties:
1. Scientific direction of the project.
2. Counselling personnel on the following procedures: a) handling of the animals, b) behavioral conditioning of the animals, c) preparation for surgery, d) performing surgery, e) post-operative care, f) recording of data, g) analysis of data.
3. Surgery.
4. Euthanasia.
5. Active participation in recording sessions.
6. Active participation in data analysis.
7. Interpretation and publication of data.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	UID:	
Phone:	[redacted]	Degree:	
Fax:		Dept:	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Rhesus Monkey

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr [redacted] is a PhD who has worked with non-human primates since [redacted]. [redacted] is experienced in most of the techniques we use and will be trained on those [redacted] is unfamiliar with by senior members of the lab.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

This person will be involved in:
1. Handling the animals
2. Behavioral training of the animals
3. Performing surgery
4. The recording, inactivation and microstimulation experiments
5. Data analysis
6. The interpretation and publication of data

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	[redacted]
Fax:		**Dept:**	[redacted]
Status:	[redacted]		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Rhesus Monkey

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Dr [redacted] is an MD/PhD who has worked with non-human primates since [redacted]. [redacted] is experienced with all of the procedures indicated in this application. [redacted] has published numerous articles in journals such as PNAS, the Journal of Neuroscience, Cerebral Cortex, the European Journal of Neuroscience and the Journal of Neurophysiology using these types of experimental preparations.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

This person will be involved in:
1. Handling the animals
2. Behavioral training of the animals
3. Performing surgery
4. The recording, inactivation and microstimulation experiments
5. Data analysis
6. The interpretation and publication of data.
7. Training junior members of the lab on the above procedures.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:		**Degree:**	
Fax:		**Dept:**	
Status:			

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Rhesus Monkey

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

[redacted] is an undergraduate student who has never worked in a lab before. [redacted] will be closely supervised and trained by senior members of the lab.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

This person will be involved in:
1. Handling the animals
2. Behavioral training
3. Observing surgery
4. Assisting in the recording, inactivation and microstimulation experiments.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

DLAM Staff

View Person Detail

Email:		**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	
Fax:		**Dept:**	DIV LAB ANIMAL MEDICINE
Status:	Staff		

What role will this person be performing in this protocol?

Personnel

Which species will this person handle in this protocol?

Rhesus Monkey

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

No

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

Please list the duties (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

DLAM Veterinary Staff perform the pre-, intra- and post-operative care of the animals in all surgical procedures.

Will this person handle radioactive materials or radioactive animals?

No

Contacts

Name:	[redacted]
Contact Type:	Emergency, Administrative
Home Phone:	[redacted]
Mobile Phone:	[redacted]

Email: [redacted]

Funding

1. Funding Types (Check All That Apply):

- ☐ Department
- ☑ Extramural
- ☐ UCLA Academic Senate
- ☐ Gift
- ☐ No funding at this time
- ☐ Other:

Proposals

List all funding agencies to which this animal protocol has been or will be submitted for consideration. Include all pending applications.

For each grant/proposal submitted to a funding agency, submit a copy of the grant proposal. If the agency is not listed, please contact the **Office of Contracts and Grants.** Please note that the National Institutes of Health may be found by typing in the keyword "NIH" when searching for an Agency Code.

Please note that the Public Health Service (PHS) Policy requires the Institution to verify approval of those components of the grant application or proposal related to the care and use of animals. **Therefore, it is strongly recommended that prior to submission, investigators review all of the proposed experiments pertaining to animals in the grant application to ensure congruence with the animal research protocol. Please detail any inconsistencies between the grant and the protocol in the spaces below.**

Agency Name: [redacted]

Agency Code: [redacted]

PI of Proposal/Award: [redacted]

Proposal/Award Title: [redacted]

Proposal/Award Number: [redacted]

Please detail any inconsistencies between the grant and the protocol in the space below (e.g., species or activities described in grant not in ARC protocol, projects completed or not begun, etc.):

Agency Name: [redacted]

Agency Code: [redacted]

PI of Proposal/Award: [redacted]

Proposal/Award Title: [redacted]

Proposal/Award Number: [redacted]

Please detail any inconsistencies between the grant and the protocol in the space below (e.g., species or activities described in grant not in ARC protocol, projects completed or not begun, etc.):

Rationale

1. Provide a non-technical summary of the overall objectives of the study.

The overall objective of this research is to clarify the mechanisms by which neurons and the networks to which they

are connected acquire and deal with visual information. Specifically, we are interested in understanding how this information is used to drive eye-movements, how it is used in perception, as well as how it is used in cognitive functions, such as attention, memory and our internal representation of visual space.

2. Indicate the possible benefits to mankind and/or animals or the advancement of knowledge that may be derived from this study.

The primary benefit of this research is to add to the growing understanding of how the brain processes information related to perception and action. The major premise is that a better understanding of neural function in normal primates will provide a basis for developing improved diagnostic and therapeutic techniques for dealing with the neurological problems of human patients. The specific issues that this work focuses on include visual attention, eye movement control, short-term memory and the representation of space. These can be affected by normal or pathological aging, or by a number of neurological and mental disorders. It is these problems that this work will help build a foundation for.

3. Explain the rationale for the use of animals, including (a) why the chosen species is the most appropriate for the study and (b) why the chosen species cannot be replaced with a phylogenetically lower species. Note that cost cannot be accepted as a justification.

(a) Our current studies can best be conducted on the Rhesus monkey for a number of reasons. First, Rhesus monkeys are highly visual animals and their visual system, as well as many other brain systems, is similar to humans, both anatomically and physiologically. Second, they are intelligent primates proven capable of learning and performing challenging behavioral tasks that test higher cognitive functions. Third, there is a large body of evidence available on this animal's behavior in cognitive tasks, as well as its neuroanatomy and neurophysiology, with particular reference to the cerebral cortex and the eye-movement centers in the brainstem.
(b) To achieve our scientific goals, it is essential that we conduct our experiments in awake, behaving subjects. They must be intelligent enough to perform the challenging behavioral tasks that we use. It is not possible to replace the rhesus monkey with a phylogenetically lower species, because they would not be able perform the behavioral tests that we use to get at the higher cognitive functions we are interested in.

Experimental Design & Justification for Requested Number of Animals

1. Provide a two- to four-sentence lay description of the experimental procedures written in language easily understandable to a seventh grade student.

We train monkeys to play simplistic video games that let us understand a bit about how they think. We then listen to the electrical activity from the cells in their brains, while they play the game. Once we think we know what the cells are doing, we can see if we are correct by turning the cells off or tickling them with electricity to see if we can change how the monkey behaves and whether it is in the way we predicted. To do these experiments, the monkeys have implants surgically placed. A monkey is usually involved in the experiments for more than 5 years, after which it is euthanized.

2. Provide a complete description of: (a) all activities involving the use of research animals; (b) a scientific justification for the total number of animals required to conduct this study. The number of animals justified in this section must match the totals in the Pain Category Assignments. To the extent possible, assign all animals to experimental groups, which can be easily distinguished by the independent variables defining each group (e.g.,drug dosages, time points, controls, etc.). Clearly indicate the number of animals needed per group and explain how group sizes were determined, either(i) by statistical analysis, or (ii) where statistics are not applicable (e.g., teaching labs, feasibility studies, antibody production, etc.), on the basis of other considerations (e.g., student/animal ratio, tissue yield per animal, antigen/animal ratio, prior experience, etc.). If statistical analysis is employed to determine the number of animals required, please specify the statistical method used.

a) Many of the experiments in the lab revolve around our hypothesis that cortical area LIP (the lateral intraparietal area, within posterior parietal cortex) acts as a priority map, which guides the allocation of covert attention and eye movement target selection. Current experiments are aimed at understanding how neurons in LIP calculate the value of objects in a scene, how this activity is used to create attentional modulation in earlier visual areas (such as areas V4 or MT) and how the output of this area is used to guide eye movements as the signal is passed to prefrontal cortex (the frontal eye field; FEF) and the mid-brain (the superior colliculus).

In all experiments, Rhesus monkeys are trained to perform tasks that require the perception of sensory information and, in some cases, the retention of that information for a short period of time (up to a few seconds). Before training, the monkeys have headholding devices and, if necessary, scleral coils and recording chambers surgically implanted under general anesthesia. For the experiments, all animals will have been trained to perform a number of behavioral tasks, including, but not limited to, simple fixation, memory-guided eye movements, match-to-sample tasks, visual search tasks, and change detection paradigms. Details of the most common tasks we use are described in the Non-Surgical Procedures section. Each animal is tested while sitting in a primate chair and facing a monitor or projection screen for presentation of visual stimuli and delivery of reward. It takes from a few days (for a simple task, like fixation) to about 14 months (for a complicated memory task) of daily training for a monkey to learn to perform the task. For the more complicated tasks, a healthy monkey treats it as a game, eager to play it not only for reward but also for the challenge. Some regulation of fluids is, however, necessary (See details and definitions of water scheduling under Animal Care question #6) before each testing session to obtain steady performance, particularly in early training. The idea of the fluid regulation is to have the animals come into the lab motivated to work for liquid rewards – these can be in the form of water or diluted fruit juice (most commonly apple juice).

If the animal was not implanted with a chamber before training, then after it has been fully trained and baseline behavioral data recorded, the animal is again surgically prepared. Under general anesthesia, one or two 20 mm circular or oval chambers are implanted on the skull over cortical areas of interest for subsequent introduction of electrodes. Between sessions, the chambers are cleaned, capped and sealed. In a given session, one of three procedures is conducted while the animal performs its task: (a) microelectrode recording of cellular activity; (b) microstimulation of a small region; or (c) chemical stimulation, with antagonists or agonists of neurotransmitters. Examples of these include Muscimol, a GABA receptor agonist, which reversibly shuts down the activity of the neurons in the inactivated area and SCH23390, a selective dopamine D1 receptor antagonist which is known to enhance

the activity of neurons in dorsolateral prefrontal cortex. The experimental procedures will be conducted remotely or prior to behavioral testing and do not distract the animal performing the task. Each animal is used for several years, although the necessity of the experimental design often has them on 'vacation' for a month or more, during which time they receive much greater volumes of water per day (80+ ml/kg/day). Until it has been established that a new animal won't gorge himself on fluid or food, water intake will not increase by more than 20 ml/kg/day in that animal, if it has been chronically provided with less fluid than 60 ml/kg/day. If all animals in a cage are on a break for more than 2 months, then they are all given free access to water via the water mains lines connected to the cage. If they go off or on water scheduling from free water consumption, then water intakes are increased or decreased gradually over a period of approximately 2 weeks.

The experiments are designed to obtain from each animal as much information as possible within the constraints stated above. Animals will usually be tested 5 days a week, with a maximum of 6 days a week. Each testing session will be preceded by a maximum 22-hour period of liquid scheduling. Some animals work in the mornings and some in the afternoon. When animals are on "training holidays" such as over the weekend, they normally receive their daily water ration in the morning. For animals that work in the afternoon following a "training holiday," we have two ways of ensuring they get access to water less than 22 hours before they work. Either we will divide the daily ration into two equal rations given in the morning and late afternoon of the "training holiday" or they will receive at least 5-10% of their daily water intake, in the form of water, in the morning of the day they will work and no more than 6 hours before they will work. For the morning top-up, the default volume will be 10% of their daily water, but this may be reduced to a volume as low as 5% of their daily water if evidence across more than 3 days shows that a greater amount of water significantly affects the willingness to work.

Because of the gradual introduction of the water scheduling, the animals show little, if any, stress for the first few days. At no time do they show significant weight loss (over 8%) or any other sign of water deficiency – as their total (weight-adjusted) daily water intake is more than adequate. The testing apparatus is cleaned and disinfected with a disinfecting detergent such as Omega. The liquid reward system is rinsed with water, and cleaned with bleach at least once a week. Primate chairs are scrubbed clean after each session with a disinfecting detergent such as Omega. The edge of the head-cap implants are monitored closely and gently cleaned and disinfected with Betadine and hydrogen peroxide. Recording chambers that are not hermetically sealed and partially filled with silicone are cleaned and disinfected (Betadine, hydrogen peroxide) before and after every recording session, and at least 3 times per week in operated animals that are not being recorded from. Hermetically sealed chambers are checked at least once a week and cleaned as necessary.

The testing will consist of a series of trials of the behavioral task under study, with each session lasting about 2-6 hours. The monkeys will be constantly monitored with a closed-circuit TV. This is just to provide live monitoring and is not recorded. At the end of testing the animal will be returned to the home-cage. The exacting tasks that we are asking our monkeys to perform over periods of months or years require a high and consistent level of motivation and, more importantly, perfect health. For this reason, we rely much on the good services of the DLAM. Further, we submit our animals to continuous inspection for signs of ill health. Each animal is watched daily for unusual or abnormal behavior, level of activity, appetite, etc. Animals active in the project are weighed at least 3 times a week, and by walking the animals to the scales we are able to monitor their strength better than by only observing them in the primate chairs or their cages. If an animal shows a sudden and consistent loss in weight, we will contact the DLAM vets and increase the volume of water the animal gets (initially to 60 ml/kg/day) and monitor the animal closely. We define a sudden and consistent loss as an 8% drop in weight over a 2 day period to a value below the mean weight (based on the previous two weeks), which remains low for 2 days when the animal is receiving 40 ml/kg/day or less. For animals receiving more than 40 ml/kg/day, we define it as a 10% drop over a 2 day period to a value below the mean weight, which remains low for 2 days. If an animal nears the 8% weight loss, it will be weighed daily until its weight stabilizes or its fluid intake is increased. Long term weight is tracked on charts on the door to the vivarium. Every day the animal is weighed, its weight is plotted on a graph with the volume of water it received beside the chart, so it is easy to see if increases or decreases in weight coincide with changes in fluid intake. The chart is checked by lab members whenever they are in the room and can be checked by DLAM staff at their convenience. When animals are on medication, they are water scheduled at 60 ml/kg/day or more. They usually receive a full bottle of water, but may receive less if they were water scheduled the previous day.

(b) We plan to use a total of 8 animals over the course of this 3 year period. We expect that we will run 6 experiments in the next 3 year period. The techniques used in each experiment will be a subset of all the techniques outlined in this protocol, as we ask different questions using the same tools. For example, all require water scheduling, surgical procedures and species restraint.

Of the six experiments, two will utilize our visual foraging task and are aimed at understanding how value is represented in parietal cortex and how this is transformed as it is passed to frontal cortex and the superior colliculus. One involves a series of simple choice tasks involved in understanding how the identity of a stimulus at the fovea affects the responses of neurons in the frontal eye field. The remaining three studies will use a new task that is a hybrid between a visual search task and a motion-direction discrimination task. We will be using this task to study how evidence for decision making accrues in parietal cortex (looking at areas MT, MST and LIP), how it is affected by focused or spread visual attention and how inactivation of the frontal eye field affects behavior and responses in these areas.

Generally, two animals are involved in a particular experiment, so we would expect to use 12 animals. However, as in the last 3 year period, monkeys will participate in multiple experiments. Each monkey usually works on a study for 4-24 months. Once that study is completed, we then use the same monkey, usually performing the same behavioral task, for a new experiment. As such a monkey who starts off as a behavioral monkey, may then join a single unit recording experiment, or a monkey who starts off in a recording experiment may then go on to a microstimulation experiment, etc. Thus, we expect that each animal will be a part of one or two experiments over the next 3 year period; so we expect to use 8 animals.

We feel it is ethically more justifiable to use the same few subjects repeatedly, especially once they are trained on complex behavioral tasks and used to the water schedule protocol, rather than using new subjects for each experiment. I expect that a significant number of the monkeys used in this protocol will continue into future renewals, just as we have kept 4 animals from the last 3-year cycle. This is one of the ways that we attempt to use the fewest total number of animals as possible in the long run.

As described in the "Surgical Procedures and Post-Operative Care" section of the protocol, we propose that a new animal will go through no more than 3 surgical procedures in their first experiment and all animals will undergo no more than 2 surgical procedures in subsequent experiments. Thus, the animals who are presently in the lab will undergo no more than 4 additional surgical procedures in the next 3 year cycle and new monkeys will undergo no more

than 5 surgical procedures in the next 3 year cycle. We note that this is a theoretical maximum and we will rarely (if ever) reach these numbers. For example, in the last year, monkey L, M and O each had one surgical procedure and monkeys K and N have not had any surgical procedures.

I hope it is clear that we do far fewer surgeries than the theoretical maximum, but in a single complicated experiment, it is possible that we will do 3 surgeries.

Generally, when animals are not on 'vacation', they are working 5 days a week. There are several reasons that we try and keep them going every day. First, when they work every day, they tend to learn tasks quicker than when the schedule is disrupted. Second, in trained animals, we notice that they tend to get into a groove if they are working every day. Third, many animals actually like to get out of their cage and work in the lab, particularly if they are working on a task they enjoy. Thus, working 5 or even 6 days a week is not seen as an additional burden over working 3 days a week, and in some cases is preferable both from our point of view and the animals' point of view.

Usually training plus data collection takes 3-24 months, then the animals go on a break for 2-6 months while the data are analyzed and written up. Breaks can also occur around the time of conferences or early on when preliminary data have been collected and need to be analyzed. There is a lot of variability that depends on how rapidly the animals learn their tasks (how difficult the tasks are), how quickly data is collected and how much data is needed. When the animals are on breaks, they often are water scheduled, ie. they get their water from a bottle at a particular time of day, however the volume is large (60-80+ ml/kg/day). In these cases, there is often water sitting in the bottle for all of the day. So while they are technically water scheduled, in practice, they do not have restricted access to water.

In my experience most animals work for more fluid than 22 ml/kg/day, we generally only use this lowest level in training when we need them to be highly motivated. Once they have learnt the task, many get 30-35 ml/kg/day. We determine this level early on during a period in which the animal is not undergoing difficult training. In this time, the amount of water the animal gets is increased slightly each day. We aim to find the maximum level of water that does not have any impact on the animal's behavior the next day. We undergo similar procedures as the animals grow, however we only do so when the animals are not actively involved in physiological recording. For example, our 2 fully trained monkeys are currently receiving, on average, 25 (monkey M) and 35 (monkey L) ml/kg/day.

This project is both a within-subject study and a between-subjects study. As a within-subject study, where the N's are cells, task-trials, conditions, etc., the study has high statistical power. That power derives from within-animal replication and the use of each animal as its own control. These factors provide internal consistency to the data, whereby conclusions for a given animal can be firmly established. However, because only a few monkeys are used, between subject comparisons have less statistical power. Consequently, we make the assumption that our observations on a few animals are valid for the general population of rhesus monkeys. This assumption is made by all researchers in the field and is widely considered a better alternative than using more animals. In addition, when experiments have built upon previous studies in the field, they typically confirm the findings of the previous studies, continuously validating the assumption.

All medications and experimental drugs are pharmaceutical-grade, except for muscimol, SCH22290, and ibotenic acid, these are all non-pharmaceutical grade no PG alternatives are available, these will be filtered through a 0.22 um filter prior to use. Please note that the volume for all compounds administered via the intramuscular route depends on the size of animal. For each drug I have provided the dose, which limits the volume given. For any drugs that I don't use often (such as post-op analgesics), I will always confer with the DLAM vets before use. Finally, after consultation with a DLAM veterinarian, I have confirmed that the 0.5 ml volume limit is set for small monkeys and that this goes up as the animal's size goes up. Specifically, we are agreed that for an 11 kg monkey, we should stay under 2 ml, and for a 5 kg monkey, we should stay under 0.5 ml. So, whether we use single or multiple injections for a single administration of a drug im will depend on the animal's size and the volume in question. Again, in practice we will confer with the DLAM vets whenever new animals come in so that we can make sure we are on the same page

Pain Category Assignments

NOTE: A painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to wh ch that procedure is applied. Examples of potentially painful/distressful procedures include, but are not lim ted to the following: terminal surgery; exuberant inflammation from adjuvants; ocular and skin irritancy testing; food or water deprivation beyond that necessary for normal presurgical preparation; noxious electrical shock that is not immediately escapable; paralysis or immobility in a consc ous animal; extensive irradiation.

Category	Description
C	Momentary or no pain/distress (Examples: injections of non-toxic substances; peripheral blood collections not requiring anesthesia; euthanasia and harvesting of tissue only; observing natural behavior; behavioral testing without signif cant restraint or noxious stimuli.)
D	Pain/distress relieved by use of appropriate anesthetics, analgesics, tranquilizers or by euthanasia (Examples: terminal surgery; survival surgery; retro-orbital blood collection; euthanasia of animals showing signs of more than slight or momentary pain and/or distress.)
E	Pain/distress can not be relieved by use of anesthetics, analgesics, or tranquilizers, as the use of these agents would interfere w th the experimental design (Examples: pain research; toxic ty testing.)

Species:	Rhesus Monkey
Strain or Breed (if applicable):	
Average Weight:	5-16 kg
Sex:	Male
Pain Category:	D
Previous Number of Animals Approved:	8
Change in Number of Animals Needed (+/-):	0
Number of Animals Needed for the 3 Year Period:	8

Pain Category

1. If the animals are listed under Pain Category D and/or E, check below all criteria that will be used to assess any potential pain/distress/discomfort in the animals. If applicable, include criteria used to evaluate post-operative pain/discomfort.

- [x] Restlessness
- [x] Vocalizing
- [x] Decreased or impaired mobility
- [x] Conjunctivitis, corneal edema, photophobia
- [x] Licking, biting, or guarding a painful area
- [x] Failure to groom, unkempt appearance
- [x] Open sores/necrotic skin lesions
- [x] Loss of appetite
- [x] Weight loss.
  Percentage weight loss (max allowable 20%): 8-10%
- [ ] Other:

2. If the animals are listed under Pain Category E, please specify the pain/distress/discomfort experienced by animals as a result of the experimental manipulations and provide scientific justification indicating why pain/distress/discomfort-relieving methods will not be employed in this protocol.

NOTE: Procedures that may cause more than momentary or slight pain or distress to the animals must be performed with appropriate sedatives, analgesics or anesthet cs, unless withholding such agents is justified for scientific reasons and will continue for only the necessary per od of time.

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E. Federal Regulations require that investigators consider alternatives (the 3 Rs - replacement, refinement and reduction) to procedures that may cause more than momentary or slight pain or distress to animals.

3. Consider all the alternatives listed below and explain why each of the following is not an available alternative for the proposed potentially painful/distressful procedure.

A. Replacement of animals with non-animal models (e.g., in vitro procedures, computer model) or a phylogenetically lower species:

Computer/mathematical models can complement, but not replace, the non-human primate. To study the subject of this project toward the achievement of its goals, no other alternatives are available. We do use computer models to simulate central processes in cognitive function. These are empirical models, based on the data from our primates, and are no substitute for them. For the reasons explained above, lower phylogenetic species are not suitable alternative for investigating the substrate and mechanisms of primate higher cognitive functions. Human subjects are also not appropriate for answering the questions we wish to ask; non-invasive procedures such as fMRI, PET or EEG/MEG do not provide information from single neurons, and do not provide both the spatial and temporal resolution we gather from the monkey. Furthermore, we cannot test the hypothesis gained from the single neuron recordings on humans, whereas with monkeys we can use reversible inactivation or electrical microstimulation.

B. Please discuss why the procedures cannot be further refined in order to minimize potential pain and/or distress to animals:

Based on current technology, there is no alternative method to implant head-caps or recording chambers (for electrodes) other than cranial surgery. To access the cortex with our probes, we need to excise a portion of the scalp and to make openings in the skull. The surgical implant of the chambers is the least invasive procedure that we have been able to devise for the purpose. The surgery is as short as we can safely and efficiently make it. Post-operative care includes the use of analgesics to minimize pain and discomfort.

Likewise, the primate chair is the only method of restraining an animal so that single neurons can be recorded from the brain while it performs a behavioral task. Its design has been refined over the last 35-40 years to optimize comfort for the animal, while allowing the experimenter to still obtain the required data.

Infrared eye-tracking methodology is a non-invasive, non-contact, and innocuous method for measuring eye position that is frequently used in humans but that is inferior to the search coil technique both in spatial and temporal resolution. We will use the technique, as described by [redacted] for behavioral tasks that do not require precise timing of eye movements, but will continue to implant coils and use the search coil technique on animals that are performing tasks in which we rely on the precise timing of the eye movements to analyze our data.

We use a water scheduling regime for our monkeys. For the behavioral tasks we use, having no reward or a reward that is not motivating, rarely allows us to collect enough data to be statistically useful. Most of the time, we use water as a reward, but some animals perform better when given diluted fruit juice (usually apple juice). One alternative is the use of a food reward. There are two main reasons we do not use this method. The first is that liquid rewards are more viable when running longer sessions; smaller quanta of reward can be given, so the animals will perform more trials. The second is that generally, experiments using a food based reward (without liquid scheduling) have only been successful with simple behaviorally tasks. Some of our tasks are complicated to learn and challenging to perform, so the heightened motivation with water scheduling is preferable.

In terms of water scheduling, we try to optimize the amount of water each animal receives on a continuing basis. When monkeys are not being trained or run over long periods of time, we slowly raise their water intake over 2-4 days. Further, monkeys generally receive more water on Fridays and Saturdays, since they usually do not work on Saturdays and Sundays. Thus, the monkeys are rarely restricted to the 22-30 ml/kg/day levels of water for more than 5 days in a row.

For behavioral training and testing, it is possible to give the animals more water and run much shorter sessions, and collect the same amount of data over more time. Indeed, I have done this in a behavior-only study [redacted] However, for the neurophysiological sessions, we need to optimize the amount of time within a single session, since neural data collected on different days cannot be directly pooled in the same analysis

behavioral data, because different neurons are collected in different sessions. Unfortunately, it is not simple to train an animal on the short sessions and then jump to long physiology sessions. Generally, if an animal has spent 6-12+ months performing 2 hr sessions, it takes many months to get the animal to work for 4-5 hrs, even with a lower water intake. Indeed, it is the fact that the animals start to get used to working for a certain period of time that allows us to increase the water given per reward once the animal is trained.

Also note that decreasing the number of times per week that they work will not decrease the amount of water scheduling. As long as the animals are working at least 1 day a week, their water intake must be controlled. There are 2 important reasons for this. First, and foremost, we reduce the possibility that the animals will go on a drinking binge and bloat (which could result in their death). Secondly, if the animals drink too much on a non-experimental day, they may be satiated when they come into the lab the next day and refuse to work (or give a much shorter session), which in the long run extends the time that they will be working on the experiment.

C. Reduction in the number of animals proposed in this application (e.g., fewer animals involved in potentially painful procedures):

As described above, we will attempt to use only 2 animals per experimental group. Occasionally, the behavior differs between 2 animals and in these cases we will require a third animal. However, this protocol is written under the assumption that 2 animals will be adequate for each experimental group. The use of only 1 animal alone for a group would not be appropriate.

Pain Literature Search

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E.

Please note that according to PHS Policy IV.C.1.a, the Guide for the Care and Use of Laboratory Animals (the Guide p. 10) and USDA Animal Welfare Act Regulat ons §2.31(d)(1)(i) "procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals." Further, in order to meet the above-ment oned regulatory requirement and in accordance with UCLA's Animal Welfare Assurance on file w th the National Institutes of Health Off ce of Laboratory Animal Welfare (OLAW), the Committee must ensure that the "principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has prov ded a wr tten narrative descript on of the methods and sources used to determine alternatives were not available." Please also note that the Comm ttee recommends the use of keywords that are specif c to the painful/distressful procedures you will be conducting and the animal model that will be used.

1. Indicate at least two databases or other sources consulted to support the conclusion that appropriate alternatives are not available.

- [x] Pubmed (Medline)
- [ ] PsychINFO
- [ ] Altweb
- [ ] UC Center for Alternatives
- [ ] Animal Welfare Information Center
- [x] BIOSIS
- [ ] Current Contents
- [ ] Other:

2. Combination of keywords used during the search:

Please specify the keywords used in the box below, including 1) the specific painful procedures that you are conducting, 2) the animal model being used and 3) alternative terms (e.g., animal model, welfare, pain, stress, distress, methods, *in vitro*).

Please see the following examples, noting that the keywords listed only apply to a protocol involving these experimental variables:

Mouse and chronic implant and in vitro model
Mouse and artery ligation and pain
Mouse and sleep deprivation and welfare

Keywords used:

BIOSIS Previews:
("nonhuman primate" OR "macaque" OR "Monkey") AND "head restraint": 26 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "chair restraint": 38 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "water deprivation": 14 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "water restriction": 4 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "fluid regulation": 1 item
("nonhuman primate" OR "macaque" OR "Monkey") AND behavior AND motivation: 388 items
("nonhuman primate" OR "macaque" OR "Monkey") AND implant: 1670 items

PubMED:
("nonhuman primate" OR "macaque" OR "Monkey") AND "head restraint": 11 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "chair restraint": 26 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "water deprivation": 16 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "fluid regulation": 0 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "water restriction": 0 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "behavior" AND "motivation": 156 items
("nonhuman primate" OR "macaque" OR "Monkey") AND "implant": 225 items

No better alternatives for restraint, surgical implantation or behavioral control were found to our proposed procedures. The total number of papers that came up in the search was 2575. This is 22 more papers than the last search, a small enough number that allows us to look over each new paper. Note the addition of extra words addressing specific alternatives (like 'pain' or 'welfare') refines a search to exclude articles that contain 1 set of keywords (such as 'water restriction') that do not contain the second keyword (such as 'welfare'). As such, the introduction of these keywords provides fewer articles. Since the number of new articles containing the keywords I

used was few enough for me to cover completely, I believe that the best way to find alternatives is not to include the keywords such as 'pain' or 'welfare'.

3. Date of most recent search (MM/DD/YYYY):

NOTE: The literature search must be updated whenever experiments that may cause potential pain or distress are proposed/modified. The literature search must also be updated at the time of each three-year renewal, and should be conducted within 2 months of submission.

09/04/2019

4. Years Covered (e.g., 1980-2019):

1926-2019 BIOSIS Previews; Pubmed - 2019

Animal Care

1. Will the experiments involve tumor formation?

The ARC requires daily monitoring of tumor growth.

No

2. Will the experiments involve chronic disease (e.g., diabetes, chronic seizures, infections with disease agents) or a chronic condition (e.g. headcaps, implants)?

Yes

3. Will the experiments involve other procedures that may lead to potential complications (e.g., surgical procedures, administration of compounds with potential toxic effects)?

Yes

4. For <u>all</u> types of experiments, if animals may experience complications, please describe the criteria for premature euthanasia below.

Infections around the head implant or eye coils, severe or chronic diarrhea with dehydration, or other nonspecific but serious conditions refractory to treatment, as determined by the DLAM veterinarian. The decision to euthanize any such affected animal will be made jointly between the PI and the DLAM veterinarian.

5. Check below all that apply to convey special animal care requirements to the responsible veterinary staff.

- ☐ Temperature Range(s)
- ☐ Humidity
- ☐ Light Cycles
- ☐ Bedding/Litter changing schedules
- ☐ Water (e.g., sterile or deionized)
- ☐ Special diet/Feeding schedule
- ☑ Deprivation of food and/or water for reasons other than surgical preparation

6. If you checked any of the boxes above, explain special care requirements in detail.

The following special care requirements will be fulfilled:

1. Enrichment of the animals' environment.

2. Daily treats, such as fresh or dry fruit, nuts, or small candy (eg. mini marshmallows or jelly beans). If candy is given, no more than 1 or 2 small pieces are given on any day and it shall be given in no more than 5 days per week. Note that monkeys, like kids, love candy, so access to it is exceedingly useful as a motivational tool.

3. Regular T.B. testing by DLAM, at least twice a year.

4. Weekly weight monitoring, or weights are taken a minimum of 3 times a week if the monkey is being water scheduled. A log of body weight is kept for all animals. If an animal is receiving 40 ml/kg/day or less and its weight nears the 8% loss level, the animal will be weighed daily until its weight stabilizes or its fluid intake increases.

5. Close daily monitoring of behavior and general health.

6. Cleaning of recording chambers every experimental day, and a minimum of 3 times per week if experiments are not being run. Chambers hermetically sealed with silicone are checked at least once a week.

7. For most of the time, most of the animals are on a water schedule regime. This means that the animals receive a set amount of water in a bottle once a day. It is important to point out that sometimes the volume of water in the bottle may be large (eg. 80 ml/kg/day), and this is more than the animals drink when on free water. Indeed, when this level of water is given, the bottle usually has some water remaining in it throughout the duration of the day. However, because it is given once a day and the maximum volume is known, we still refer to this as a 'water scheduled' animal.

When running behavioral experiments, the animals are given a minimum water-allowance of 22 ml/kg/day everyday (7 days a week). The 22 ml/kg/day is a minimum and includes the amount of water or diluted fruit juice the animal receives during training. However, the actual amount is individualized; if an animal will work for a larger amount of water, and most work for 30 ml/kg/day or more most of the time, then the animal will get that larger amount. Before each testing session, the animal will have restricted access to water for a maximum period of 22 hrs. Some animals work in the mornings and some in the afternoon. When animals are on "training holidays" such as over the

weekend, they normally receive their daily water ration in the morning. For animals that work in the afternoon following a "training holiday," we have two ways of ensuring they get access to water less than 22 hours before they work. Either we will divide the daily ration into two equal rations given in the morning and late afternoon of the "training holiday" or they will receive at least 5-10% of their daily water intake, in the form of water, in the morning of the day they will work and no more than 6 hours before they will work. For the morning top-up, the default volume will be 10% of their daily water, but this may be reduced to a volume as low as 5% of their daily water if evidence across more than 3 days shows that a greater amount of water significantly affects the willingness to work.

Any day the animal is not being tested, it will receive, in addition to the 22 ml/kg/day, a supplement of water to bring the total per day to the average total received on days when it is tested (eg. up to 30 ml/kg/day). If the animal is not to be tested the next day, it will receive a greater volume of water (usually 40-60 ml/kg). A daily log of water consumption is kept for all animals.

Animals are usually given the opportunity to work to fluid satiety (which is usually less than their daily fluid intake), but in some instances the experiment will be stopped before they are ready to stop working. In both cases, the animal is given additional fluid in the vivarium to bring its water intake up to its normal daily value. So if an animal receiving 350 ml/day drinks 250 ml in the lab, it will be given an additional 100 ml in its cage later in the day.

Requiring the animal to perform a task for which it receives a fluid reward is not unlike conditions in the wild, in which animals must forage, travel distances, solve problems, or otherwise work to obtain their water. Our monkeys are provided with regular and more than sufficient water every day throughout the week (eg. 30 ml/kg/day is the equivalent of an average man drinking 2.7 quarts of water a day). Weight stability and task performance indicate that our water schedule is not only physiological, but also behaviorally sound.

8. In the lab, animals receive their liquid rewards via a Watson-Marlow pump. The bottle, tubes and mouthpieces (1 per animal) are cleaned after each session and sanitized at least once a week or more often if needed. The latter involves filling the entire system with a diluted bleach solution, leaving it for half an hour and then rinsing at least 3 times. If solids are seen in the tube, it is replaced. If solids are seen in the mouthpiece or water bottle, then they are removed and scrubbed with a disinfecting detergent. Cleaning and bleaching the system are separately documented on a chart on the door of the rig.

7. Environmental Enrichment: UCLA vivarium staff provide environmental enrichment to all species (please refer to the **ARC Policy on Environmental Enrichment**).

a. If you request to provide additional or alternative environmental enrichment, please describe the environmental enrichment below.

b. Please provide scientific justification if your research precludes the use of environmental enrichment.

We aim to pair-house the monkeys as much as possible, however there are a number of factors that sometimes preclude this from occurring.

1) When we have an odd number of animals, we are not able to pair-house one animal.

2) When an animal is required to be on main-line water per veterinary prescription and the other animal in the quad is being water scheduled, we are unable to pair the animals.

3) For a period after surgery, we do not pair the animals until the recovering animal is ready.

4) When both animals are water scheduled, but one is getting a large volume (such as 800 ml or a full bottle), we pair house the animals for less time than normal - this allows the animal getting more water to have more time to drink the water.

5) When the animals are incompatible and pairing them would risk injury to one or both.

6) Attempting pairing can be stressful, sometimes distressful, to an animal, and therefore we avoid this process when an animal is actively enrolled in data generation that could be affected by this.

7) When a new animal is brought into the housing room, the hierarchy in the room may change. In this case we may have to separate the animals for a more prolonged time. However, we attempt to get animals back together as soon as possible.

8) When animals are receiving medications that affect their routine behavior, we may have to separate the animals for longer periods than normal.

In all cases, the timing of attempted pairing is determined by communication between DLAM and the laboratory.

8. If you will be using transgenic animals in this research, please clarify whether there are any anticipated or suspected phenotypes of the transgenic mice that might cause pain or discomfort to the animals. If any pain, distress, or morbidity is associated with the phenotypes of this line, please indicate the criteria for premature termination of these mice.

9. PLEASE COMPLETE IF YOU HAVE MICE AND/OR RATS IN DLAM-MANAGED FACILITIES. Please check one response to the following:

I request that the veterinarian (or his/her designee) euthanize animals found to be sick or injured for me:

- ○ I request that the DLAM veterinarian (or his/her designee) euthanize my animals for me in accordance with his/her veterinary discretion at the time that they are found sick or injured. This decision will only apply to animals in cages that I've marked with a green euthanasia sticker on the cage card. DLAM will notify me of the euthanasia by email after the fact.

I understand that I remain responsible for monitoring of my animals, in accordance with my approved protocol and with the ARC Policy on ***Responsibility for Monitoring Laboratory Animals***.

I will treat or euthanize animals:

○ I assure the ARC that I will promptly respond to Veterinary Health Case notifications regarding my animals, as required by the ARC Policy on ***Notification of Investigators with Sick or Injured Animals***.

Locations

Please indicate ALL locations where animals will be housed and/or used, including:

1. **Vivarium Housing** (where animals will be housed). Please note that if vivarium housing has not been assigned, select "VIVARIUM" as the building name and "Unassigned" as the room number.
2. **Study Area** (any investigator-maintained facility outside the vivarium where USDA-covered species will be housed for per ods longer than 12 hours, or where non-USDA-covered species will be housed for per ods longer than 24 hours).
3. **Research Area** (where non-surg cal activities, including euthanasia, will be performed).
4. **Surgery Area - Survival** (where recovery surgery will be performed).
5. **Surgery Area - Non-Survival** (where terminal surgery will be performed).

Building	Room	Species	Location Type
[redacted]	[redacted]	Rhesus Monkey	Research Area **Reason:** [redacted] facil ty
[redacted]	[redacted]	Rhesus Monkey	Vivarium Housing
[redacted]	[redacted]	Rhesus Monkey	Vivarium Housing
[redacted]	[redacted]	Rhesus Monkey	Research Area **Reason:** All the experimental procedures that will be done in [redacted] may be done in [redacted]. This includes implant cleaning, behavioral training, single un t recording, reversible inactivat on and microstimulation. No work will be performed in this room until t is approved for use by the IBC.
[redacted]	[redacted]	Rhesus Monkey	Surgery Area - Survival
[redacted]	[redacted]	Rhesus Monkey	Research Area **Reason:** In this room we clean the implants, perform behav oral testing, do neural recordings, m crostimulation and reversible inactivation.
[redacted]	[redacted]	Rhesus Monkey	Surgery Area - Survival
[redacted]	[redacted]	Rhesus Monkey	Surgery Area - Non-Survival **Reason:** Room used for Perfusion.

Medications and Experimental Drugs

List below all medications/drugs/compounds/agents/etc. that will be given to the animals. Please be sure to include analgesics, anesthetics, antibiotics and all experimental drugs or treatments. Cell lines injected in suspension should be listed here.

The select on of the most appropriate med cation/agent should reflect that which best meets clin cal and humane requirements w thout compromising the scientific aspects of the research protocol. In accordance w th federal regulat ons, consultat on with an attending veterinarian is required in the planning of a research protocol involving procedures that may cause more than momentary or slight pain or distress to the animals. The **ARC Policy on Use of Pharmaceutical-Grade Compounds** requires that investigators use pharmaceut cal-grade compounds whenever they are available, even in acute procedures.

If pharmaceut cal-grade preparations are not available, please dentify wh ch compounds are affected and provide supporting justification in your Experimental Design. All non-pharmaceutical-grade drugs must be filter-sterilized pr or to use.

Please do not list euthanasia drugs in this section.

Drug/Compound Name:	Buprenorphine
Species:	Rhesus Monkey
Medication Type:	Analges c
Dose or Concentration:	0.01-0.03 mg/kg
Volume:	up to 2 ml in larger animals
Frequency:	From every 4 hrs to twice daily
Route:	other: iv or im
Length of treatment/administration:	2 to 3 days and then as needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Buprenorphine-SR
Species:	Rhesus Monkey
Medication Type:	Analges c
Dose or Concentration:	0.12-0.16 mg/kg
Volume:	
Frequency:	Once every 3 days
Route:	sc
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Atropine
Species:	Rhesus Monkey

Medication Type:	Anesthet c
Dose or Concentration:	0.02-0.05 mg/kg
Volume:	1 ml max
Frequency:	Once before surgery, given w th Ketamine
Route:	im
Length of treatment/administration:	E ther pre-operatively or intra-operatively to control bradycardia or salivation if necessary
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Bupivacaine
Species:	Rhesus Monkey
Medication Type:	Anesthet c
Dose or Concentration:	0.5-1 mg/kg
Volume:	
Frequency:	To be given w th lidocaine before surgery at the site of pin insertion or skin incision
Route:	sc
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Dexmedetomidine
Species:	Rhesus Monkey
Medication Type:	Anesthet c
Dose or Concentration:	0.007-0.03 mg/kg
Volume:	
Frequency:	Given with Ketamine
Route:	other: im or iv
Length of treatment/administration:	Once before surgery
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures

Drug/Compound Name:	Isoflurane
Species:	Rhesus Monkey
Medication Type:	Anesthet c
Dose or Concentration:	1-2.5%
Volume:	1-2 liters of O2 per minute flow
Frequency:	At time of surgery
Route:	inh
Length of treatment/administration:	Time of Surgery
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures

Drug/Compound Name:	Ketamine
Species:	Rhesus Monkey
Medication Type:	Anesthet c
Dose or Concentration:	1-15 mg/kg
Volume:	2 ml max
Frequency:	Once before surgery
Route:	im
Length of treatment/administration:	Once before surgery
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures

Drug/Compound Name:	Lidocaine
Species:	Rhesus Monkey
Medication Type:	Anesthet c
Dose or Concentration:	1-4 mg/kg
Volume:	
Frequency:	To be given w th bupivacaine before surgery at the s te of pin insert on or skin incis on
Route:	top cal
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Propofol
Species:	Rhesus Monkey
Medication Type:	Anesthet c
Dose or Concentration:	200-600 ug/kg/min or 1-5 mg/kg
Volume:	
Frequency:	continuous rate infusion or bolus for anesthet c induction
Route:	iv
Length of treatment/administration:	Time of MRI scan

Purpose:	Pre-Operative/Intra-Operative Other: To anesthetize monkey for MRI scan or to induce anesthesia for surgery

Drug/Compound Name:	Sevoflurane
Species:	Rhesus Monkey
Medication Type:	Anesthet c
Dose or Concentration:	2-5%
Volume:	1-2 liters of O2 per minute flow
Frequency:	At time of surgery
Route:	inh
Length of treatment/administration:	At time of surgery
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Cefadroxil
Species:	Rhesus Monkey
Medication Type:	Antib otic
Dose or Concentration:	20-30 mg/mk
Volume:	
Frequency:	2 times a day
Route:	oral
Length of treatment/administration:	3-5 days post-surgery
Purpose:	Post-Operative

Drug/Compound Name:	Cefazolin
Species:	Rhesus Monkey
Medication Type:	Antib otic
Dose or Concentration:	20-30 mg/kg
Volume:	~ 1 ml
Frequency:	At surg cal induction, every 90-180 min during surgery (iv) and/or 2 times a day post-op
Route:	other: im or iv
Length of treatment/administration:	During surgery and/or 3-5 days post-operative
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Ceftriaxone
Species:	Rhesus Monkey
Medication Type:	Antib otic
Dose or Concentration:	25-50 mg/kg
Volume:	
Frequency:	once or twice a day
Route:	other: iv (at induction) or im
Length of treatment/administration:	7-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Cephalexin
Species:	Rhesus Monkey
Medication Type:	Antib otic
Dose or Concentration:	20-30 mg/kg
Volume:	
Frequency:	2 times a day post-op
Route:	oral
Length of treatment/administration:	3-5 days post-surgery
Purpose:	Post-Operative

Drug/Compound Name:	Triple antib otic ointment
Species:	Rhesus Monkey
Medication Type:	Antib otic
Dose or Concentration:	5000u polymyxin, 400 Bac tracin, 3.5 mg Neomycin/gr
Volume:	Small dab
Frequency:	When implant shows early signs of infect on
Route:	top cal
Length of treatment/administration:	For 1 week or until infect on is gone, whichever is longer
Purpose:	Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Triple antib otic ophthalm c ointment
Species:	Rhesus Monkey

Medication Type:	Antib otic
Dose or Concentration:	N/A
Volume:	N/A
Frequency:	Once to tw ce daily in the implanted eye up to 7 days post-op
Route:	top cal
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Triple antib otic ophthalm c ointment w th hydrocortisone
Species:	Rhesus Monkey
Medication Type:	Antib otic
Dose or Concentration:	N/A
Volume:	N.A
Frequency:	Once to tw ce daily in the implanted eye up to 7 days post-op
Route:	top cal
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Atipamezole
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	1-2 times the volunter of dexmedetomidine
Volume:	
Frequency:	Given after Ketamine/dexmedetomidine to reverse the dexmedetom dine
Route:	im
Length of treatment/administration:	
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures

Drug/Compound Name:	Betadine
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	10%
Volume:	Small volume to cover affected area.
Frequency:	After each recording sess on, or every work day, whichever is more often
Route:	top cal
Length of treatment/administration:	For life of implant
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Bicuculline
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	5 ug/ul
Volume:	5 ul
Frequency:	No more often than once every 3 recording days
Route:	other: via m crosyringe intracerebrally
Length of treatment/administration:	Duration of stimulation study (2-3 months)
Purpose:	Other: Chemically stimulate neurons of interest

Drug/Compound Name:	Carprofen
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	2-5 mg/kg
Volume:	~0.8 ml
Frequency:	once per day
Route:	other: im or orally
Length of treatment/administration:	2 days following major surgical procedures
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Dexamethasone
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	1-2 mg/kg
Volume:	~ 1 ml
Frequency:	Given once if dura is pierced in surgery

Route:	other: iv or im
Length of treatment/administration:	Note that administrat on of carprofen or any nonsteroidal anti-inflammatory agent is contraind cated
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Granulex-V
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.12mg/g Trypsin; 87mg/g Balsam Peru; 788mg/g Castor Oil
Volume:	N/A
Frequency:	When wound edge shows signs of crusting
Route:	other: spray
Length of treatment/administration:	
Purpose:	Post-Operative Non-Surgical Procedures

Drug/Compound Name:	halobenzazepine (SCH23390)
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	5 mg/ml
Volume:	0.85 ul
Frequency:	No more than once every 3 recording days
Route:	other: via m crosyringe intracerebrally
Length of treatment/administration:	Duration of study (2-3 month)
Purpose:	Other: Pharmacolog cal activation of neurons under study

Drug/Compound Name:	Hydrogen Peroxide
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	3%
Volume:	Small volume to cover affected area
Frequency:	Before and after recording, or at least 3 times a week, wh chever is more often
Route:	top cal
Length of treatment/administration:	Lifetime of implant
Purpose:	Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Iboten c Ac d
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	10 ug/ul
Volume:	5 ul
Frequency:	Once in 3 or 4 sites
Route:	other: via m crosyringe intracerebrally
Length of treatment/administration:	
Purpose:	Other: Create perminant small lesion for identif cat on of recording sites

Drug/Compound Name:	Magnevist
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.2 mM/kg
Volume:	
Frequency:	At begining of MRI scan
Route:	iv
Length of treatment/administration:	Duration of MRI scan
Purpose:	Other: MRI contrast agent

Drug/Compound Name:	Midazolam
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.1-0.2 mg/kg
Volume:	~ 1 ml
Frequency:	At time of MRI scan
Route:	im
Length of treatment/administration:	Time of MRI scan
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures

Drug/Compound Name:	Muscimol

Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	5 ug/ul
Volume:	5 ul
Frequency:	Once every 3 recording days
Route:	other: via m crosyringe intracerebrally
Length of treatment/administration:	Duration of inactivat on study (2-3 months)
Purpose:	Other: Reversible inactivat on of neurons under study

Drug/Compound Name:	Nair
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	Stock commercial product
Volume:	Small volume to cover affected area
Frequency:	Once before surgery
Route:	top cal
Length of treatment/administration:	Once before surgery
Purpose:	Pre-Operative/Intra-Operative

Euthanasia

For each species used, please provide the euthanasia information. Techniques for euthanasia must follow guidelines established in the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

1. **Species:**

   Rhesus Monkey

2. **How will animals be euthanized?**

   Non-Physical Method

3. **For animals that will be euthanized by a physical method, please indicate that method (decapitation or cervical dislocation).**

   a. **Please indicate the appropriate physical method.**

   Other:Euthanasia will be confirmed by a lack of heartbeat and respiration for at least 10 min

   b. **Will anesthesia be used prior to use of the physical method of euthanasia?**

   c. **If anesthesia cannot be administered, please provide scientific justification.**

4. **For animals that will not be euthanized at the end of the study, please indicate the final disposition.**

Euthanasia Medications

List the drug(s) used for euthanasia on an animal by physical or non-physical methods.

Please note that according to the **AVMA Guidelines for the Euthanasia of Animals: 2013 Edition**, "compressed CO_2 in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable."

Drug Name:	Veterinary grade pentobarbital
Species:	Rhesus Monkey
Dose or Concentration:	100-150 mg/kg
Route:	iv
Purpose of Drug:	Anesthesia, Euthanasia

Tissue Collection

Please enter the following information regarding tissue collection for the protocol. See ARC Policy on Blood Collection from Laboratory Animals.

1. **Tissue To Be Collected:**

   ☐ Blood

   ☑ Other Collected: Brain

2. Frequency of blood and/or other tissue collections:

At termination.

3. Volume of blood and/or other tissue collected per time point:

Brain at termination

4. Describe techniques that will be used to collect blood and/or other tissue.

At the termination of participation in the experimental studies, an animal will be anesthetized with pentobarbital. The animal will then be perfused through the heart with normal saline followed by histological fixative solutions such as a formaldehyde solution.

5. Describe how anemia and infection will be prevented.

1. Satisfactory nutrition.
2. Topical antiseptics after each recording session.
3. Topical or systemic antibiotics if there is any indication of an infection.

Surgical Procedures and Post-Operative Care

Please complete the following questions, noting that any requested exception to ARC Policy must be justified in the space provided.

Note: ARC pol cy requires investigators to employ the following measures to ensure asepsis while conducting survival surgery: asept c surg cal techniques; asept c surg cal field; sterile instruments; clean lab coat/surg cal gown; and sterile surg cal gloves. *For information on surgeries on rodents and birds, please see the **ARC Policy on Survival Surgery in Mice, Rats and Birds***.

Non-survival surgeries of extended durat on or procedures otherwise likely to increase the risk of Intraoperative infection and/or sepsis (e.g. gastrointestinal surgery) will be evaluated on a case-by-case basis to determine whether aseptic techn ques must be used. Refer to the **ARC Policy on Non-survival Surgical Procedures** for further informat on.

Please note that surgical records are required for all animals. These records must include anesthetic administration and intra-operative mon toring, as well as post-operative recovery observations, including administration of analgesics and antib otics and suture/staple removal if appl cable. Additionally, any adverse outcomes must also be recorded.

1. Pre-Operative care will include (check all that apply):

- [x] Lab tests
- [ ] Conditioning
- [x] Fasting: 12 hrs
- [x] Other:

CBC, sed rate, hematocrit.
For major surgeries, Animals on a water schedule will be brought up to at least 60 ml/kg for at least 3 days before surgery and 2 weeks after surgery.

Please note that a phys cal examinat on is required.

2. Will neuromuscular blocking agents be used (e.g., Pancuronium, Succinylcholine)? Refer to the ARC Policy on Neuromuscular Blocking Agents.

No

3. Select all criteria that will be used to assess the proper level of anesthesia.

The level of anesthesia should be assessed on a continuous basis.

- [x] Respiration rate
- [x] Heart rate
- [ ] EEG
- [x] EKG
- [x] Muscular relaxation
- [x] Positive toe pinch
- [x] Corneal reflex
- [x] Color of mucous membranes
- [x] Other:

Animal vital signs will be monitored and recorded every 15 minutes during anesthetic procedures.

4. Surgical preparation of all mammalian species must include:

1) Removal of hair w th #40 clipper blade in a wide margin around the incis on site.
2) Three alternating scrubs using a germ cidal scrub and 70% alcohol.
3) Placement of lubricating ointment into the eyes.
4) Covering the animal except the surgery site w th a sterile drape.
5) Placing the animal on an external heat source (water circulating heat pad or heating pad set on "low" with a barrier placed between the animal and the heating pad).

- (•) I assure the ARC that surgical preparation will be performed as outlined above.
- ( ) *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

PLEASE NOTE: Any deviation from the policies above must be detailed and scientifically justified in the space below.

5. Indicate the methods to be employed to prevent (a) hypothermia and (b) dehydration (including volume of fluids and route). If this question is not applicable to the proposed surgical procedures, provide a brief explanation.

To prevent hypothermia, the veterinarian recommends the use of water-circulating heading pads over heating lamps and/or electr cal heating pads. The use of heating lamps is strongly discouraged. If not used properly, heating lamps and electrical heating pads may cause thermal injury to the animal. Therefore, describe precaut ons taken to prevent hyperthermia.

Circulating warm water pad.
Warm i.v. drip of physiological solution.

6. Surgical preparation of the surgeon must include:

1) Wash hands w th germicidal soap.
2) Sterile gloves.
3) Surgical Mask.
4) Cap and booties (not required for mice and rats)
5) Sterile gown (clean lab coat or gown acceptable for m ce and rats)

(•) I assure the ARC that surgical preparation will be performed as outlined above.

( ) *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

7. Instrument preparation must be performed by:

1) Autoclave sterilization or ethylene oxide (gas) sterilizat on.
2) Either chemical disinfect on (acceptable between multiple surgeries in mice, rats, and non-mammalian species) or
3) Hot bead sterilizer.

(•) I assure the ARC that instrument preparation will be performed using one of the methods outlined above.

( ) *Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.*

8. Duration of Surgical Procedures (Must be completed as applicable):

For non-survival surgery, ind cate the duration from anesthesia induction to euthanasia. For survival surgery, ind cate the durat on from anesthesia induction to recovery from anesthesia.

Survival: 3-8 hrs
Non-Survival: < 45 min

9. Provide scientific justification for performing multiple survival surgeries on a single animal.

Multiple survival surgeries will be approved only when they are related components of the experimental design.

We propose a maximum of 3 major surgeries on a new animal in their first experiment and 2 surgeries per animal per subsequent experiment. If more surgeries are planned, permission will be sought from this committee, unless the surgery is an emergency, one whose delay will cause pain or suffering for the animal, in which case the surgery will done with the approval of the veterinary staff alone. It is most likely that animals will undergo far fewer than the maximum number of surgeries (for evidence of this, see the end of this section).

Almost all of our experiments require the surgical placement of a head-cap and scleral eye coils (to immobilize the head and record eye movements) and many experiments require the surgical placement of a recording chamber. For experiments in which the behavioral training will be quick and neural recordings will be made, all three can be surgically placed in one surgical procedure. Because the recording chambers require far greater maintenance because of the exposed dura, there is substantial benefit from postponing the implantation of the chambers as long as possible. Thus, for behavioral experiments or experiments in which neural recordings will be made, but in which the behavioral training could take many months, we surgically implant the head-cap and eye coils first and perform a second surgical procedure to implant the chamber or chambers at a later date, when the training has been completed and/or the critical baseline behavioral data has been obtained. Generally these surgeries will be 6-10 months apart. In some experiments, the use of an additional chamber may become necessary after recording from the original chamber(s). In these cases its implantation may be deferred to a later date, and always 6-18 months after the previous major surgery.

There is one circumstance in which additional surgeries may be needed and then only with the concurrence with the veterinarian. Occasionally eye coils and or head-caps break or crack and need to be replaced or repaired in order to ensure that the implants are functioning safely and properly. Because the statistical strength in our experiments comes from within animal comparisons, an experiment cannot continue without these repairs.

The first surgical procedure a new animal goes through is the placement of the head-cap and eye coil (and chamber(s) if appropriate). If, as outlined above, the animal needs a chamber placed or a second chamber placed, the animal could undergo a second or third surgical procedure. As such, I have proposed a maximum of 3 surgeries for a new animal for their first experiment. Usually, the implants remain intact across experiments, so when an animal goes on to a new experiment, the most likely thing is that they will not need any new surgical procedures. However, if the new experiment involves the animal performing the same task, but recording from a different region of the brain, then it is possible that two more surgical procedures could be performed - one to place the first chamber in the new location and a second to place a new chamber on the other side of the head. As such, I have proposed a maximum of 2 surgeries per animal for subsequent experiments. By using animals in multiple experiments we minimize the total number of animals used by moving trained monkeys from one experimental group to another. Generally, an animal participates in no more than two experiments within the 3 year protocol (many participate in just one), so although the maximum number of surgeries is listed as 5, most will go through far fewer.

We currently have 5 animals in the lab under this protocol, as of June 2019, they have undergone the following procedures since they arrived in the lab:

- Monkey K: 4 surgeries (eye coil + head-cap + chamber; new chamber placement; moved chamber; replaced head-cap) + 1 emergency surgery since 2014.
- Monkey L: 1 surgery (head-cap only)
- Monkey M: 2 surgeries (eye coil + head-cap; chamber placement) + 3 emergency repairs since 2014.
- Monkey N: has not undergone any surgical procedures
- Monkey O: 1 surgery (head-cap only)

Based on the scientific justification for the number of surgeries per animal per experiment given in the previous paragraph, the animals who are presently in the lab will undergo no more than 4 additional surgical procedures in the next 3 year cycle (2 surgeries per experiment and a maximum of 2 experiments per animal) and new monkeys will undergo no more than 5 surgical procedures in the next 3 year cycle (3 surgeries for the first experiment and 2 surgeries for those who may participate in a second experiment). We reiterate that this is a theoretical maximum and we will rarely (if ever) reach these numbers. For example, in the last protocol period, we performed a total of 5 surgical procedures on four different animals, with no more than two procedures on any one animal. However, if we think additional surgeries above the maximum are required, we will consult with the DLAM veterinarians prior to proceeding.

10. Please describe all surgical procedures, including non-survival procedures.

All surgery will be conducted under strictly aseptic conditions, with the animal under general anesthesia and mounted in a stereotaxic instrument and animals receive analgesics before and after surgery. After major protocols, animals are also given a NSAID and antibiotics. As a general rule, we have the DLAM veterinary staff perform all drug-related duties (pre-op preparation, anesthesia and animal monitoring during the procedure, and post-op care) and the surgical preparation. Members of the lab perform the surgical procedure under the PI's supervision, under Dr [redacted] supervision and/or under the supervision of a vet, if neither the PI nor Dr [redacted] is available.

Medications for Surgical Procedures:
Buprenorphine, buprenorphine-SR, carprofen, bupivacaine and/or lidocaine will be used as analgesics, as appropriate for the individual animal and surgery. Ketamine, dexmedetomidine, diazepam, midazolam, xylazine, isoflurane, sevoflurane and/or propofol will be used as induction or maintenance agents as appropriate for the individual animal and surgery. Cefazolin will be used as a surgical prophylactic antibiotic if the surgery does not involve the skull or dura. Ceftriaxone will be used as a surgical prophylactic antibiotic if the surgery does involve the skull. Atropine is given as needed to control salivation or bradycardia.

Surgical Preparation:
Because of the relatively large size of our animals, the stereotaxic instrument is attached to the operating table and its height adjustable. Anesthesia will first be induced with ketamine and Xylazine, after which animals will be prepared in an adjacent room. The surgical site will be prepared by removing any hair from the area with clippers followed by a depilatory (Nair, CaOH) if necessary. The area will then be scrubbed with Betadine a minimum of three times, washed with 70% EtOH and then painted with Betadine solution. After that, the animal is transported to the O.R., trachea intubated, i.v. line introduced, surgical field delimited, and draped with sterile blanket. Vital signs are continuously monitored. Body temperature is thermostatically maintained by circulating warm water pad or air heater.

Eye Coil Procedures:
If the scleral coils used to record eye movements are not to be implanted, bland ophthalmic ointment will be applied to both eyes following administration of anesthesia to prevent desiccation, otherwise, they will be implanted using a special technique that has been described in the literature (see Judge, Richmond & Chu (1980)) and is used in many laboratories around the world studying the oculomotor system in awake monkeys. After opening the eye lid with a speculum, an incision is made in the limbus connecting the conjunctiva to the eye, using fine micro-dissection scissors. Q-tips are then used to blunt dissect under the conjunctiva, and, once open, the coil is placed around the eye. A large needle (1/2 curved suture needle, size 4) is then used to pull the wires from the coil around the orbit and out of the skin on the forehead. The wire is then reinserted subcutaneously and is brought out at the location of the head-cap, where it is attached to the plugs once the head-cap placement is complete (see below). Once a coil has been implanted, the ophthalmic ointment is put on the eye.

Head-cap and Chamber Placement:
Following the implantation of coils around one or both eyes, the animal is adjusted in the stereotaxic device to ensure the correct orientation of the head and to facilitate the correct placement of the remaining implants: the head-cap and recording chamber(s), which are made of high-grade stainless steel, titanium, PEEK or a copolymer resin (20% glass-filled Delrin 570). A midline incision will be made through the skin over the cranium extending from the region of the external occipital protuberance to within a centimeter or two of the brow ridges. If necessary, the temporalis muscle will be reflected or partially removed to gain access to the underlying periosteum. The periosteum will then be cleaned and dried. In animals that already have a head-cap, the viability of the proposed site for the chamber on the skull will be determined by the PI or a senior lab member in consultation with a DLAM vet. When placing chambers, a trephine will be used to remove a circular disc of bone from the cranium, taking care to ensure that the underlying dura is left intact. In the unlikely event that the dura is damaged sufficiently to allow herniation of cortex, the craniotomy is replaced, the groove filled with bone wax and, if possible, the entire area sealed with dental acrylic. Cranial implants will be held in place with one of two methods: 1) a titanium headholder with 2-4 adjustable strips will be positioned stereotaxically, and the strips screwed to the skull; or 2) a dental acrylic (methyl methacrylate) cap will be secured to the skull with numerous stainless steel, titanium or ceramic screws. After a final cleaning with saline and/or hydrogen peroxide, the periosteum will be dried and may be painted with cyanoacrylate (Vetbond). A stainless steel, titanium, or copolymer resin plug (used to secure the head to the primate chair during experiments) will be positioned stereotaxically and then secured to the skull with dental acrylic, which bonds together all of the screws/bolts/strips to form a single robust implant. Edges of the acrylic will be made as smooth as possible, using Teflon sheets if appropriate, to minimize wound edge irritation. Recording chambers will be positioned over the trephine hole stereotaxically and secured to the rest of the implant with dental acrylic, or, in the case of the titanium headholder, four nylon screws will be implanted in the skull using key slots, and the chambers bonded to them by acrylic. The wound edge will be cleaned and sutures (skin staples or non-absorbable 2-0 or 3-0 sutures) inserted to keep the skin closely opposed to the implant until healing has occurred. If necessary, modified V-plasty incisions will be used to ensure that the skin is closely aligned with the margin of the implant.

Perfusion:
For perfusions, the animals will be given a high dose (100-150 mg/kg) of veterinary grade pentobarbital. After

verifying the absence of pedal (toe-pinch reflex), an incision will be made along the midline of the chest and the diaphragm will be cut back and the ribs opened to expose the heart. A small cut will be made in the left ventricle through which a tube with a thick glass end piece will be inserted up and into the aorta. Thin aseptic string will be used to secure the end piece in the aorta. The right atrium will then be cut and the descending aorta clamped. A pump or gravity feed will be used to perfuse the upper body with isotonic saline, followed by Formaldehyde.

11. Please indicate the suture materials to be used:

- [x] Internal: absorbable sutures (e.g., Dexon, Vicryl)
- [x] External: non-absorbable skin sutures (e.g., Nylon, wound clips). *Please note that external skin sutures or wound clips must be removed 7-14 days following surgery.*
- [ ] Other/not applicable (describe below):

12. During recovery from anesthesia, what indications will be monitored to assure the animals are stable?

In accordance with the Guide for the Care and Use of Laboratory Animals, particular attent on should be given to thermo-regulation, card ovascular and respiratory function, and post-operative pain or discomfort during recovery from anesthesia.

Respiration, heart-rate, mucous color, mobility, eye reflexes and temperature are monitored continuously and recorded every 15 min until just before extubation. The animal is placed back into its transport cage upon extubation. After extubation, and until the animal is able to climb onto the perch, the animal is monitored continuously for signs of pain, dysphoria, nausea, or abnormal ambulation or respiration. The DLAM vet is alerted if any abnormal signs are seen.

13. How often will animals be monitored after anesthetic recovery?

The ARC requires that animals be observed continuously by trained personnel during the immediate anesthet c-recovery period (i.e., until the animal is ambulatory) and at least daily after anesthetic recovery. However, post-operative mon toring frequency may be greater depending on the complex ty of procedures involved, administration of post-operative analgesia, and the species of animal used.

The animal is monitored continuously, with observations recorded every 15 min, until it is able to climb up onto a perch, and then at least daily thereafter for 3 days following a non-invasive procedure (such as implant maintenance) or for 10 days if the animal has sutures.

Species Surgery

Species:	Rhesus Monkey
Number of Animals:	8
Surgery Type:	Nonsurvival Surgery
Surgeries per Animal:	1
Time Between Surgeries:	

Species:	Rhesus Monkey
Number of Animals:	4
Surgery Type:	Multiple Survival Surgery
Surgeries per Animal:	4
Time Between Surgeries:	6-18 months

Species:	Rhesus Monkey
Number of Animals:	4
Surgery Type:	Multiple Survival Surgery
Surgeries per Animal:	5
Time Between Surgeries:	6-18 months

Non-Surgical Procedures

1. Describe the basic methods used for all non-surgical manipulations (e.g., imaging, behavioral studies, Parkinson's and diabetes induction, chronic implant maintenance, cannulation).

There are 10 non-surgical procedures.

1. Implant maintenance. All instruments and consumables used in this process are pre-sterilizes, heat sterilized (using a sterilizing over) or liquid sterilized (using a safe agent such as Cidex OPA).

(a) Headcap maintenance. Routine care will consist of gentle cleaning of the skin-implant margin with saline or a dilute Betadine solution. If crusting occurs at the margin, products such as Granulex-V (Beecham) may be used to assist wound healing through debridement and stimulation of epithelial growth.

(b) Recording chamber maintenance. We have 2 main forms of recording chamber maintenance: Standard cleaning and Silicone Sealing. Usually, animals with recording chambers undergo the standard cleaning procedure. Sealing is generally used when the chamber is conducive to creating a good seal (such as, whether it is hermatically sealed or whether the dura is tangential to the edge of the chamber) and the animal will be on a break from recording or has just undergone placement of both a head holder and recording chamber.
(i) Standard cleaning. Chambers are flushed with hydrogen peroxide, rinsed multiple times with sterile saline and then flushed with diluted Betadine and rinsed again with sterile saline. The walls of the chamber are cleaned by [redacted]

with a Q-tip and the cap is cleaned separately with hydrogen peroxide, a Betadine scrub solution and is then sterilized. Chambers are always sealed with a pre- sterilized cap. If the chamber is used for physiology, it is cleaned both before and after the session.
(ii) The use of a surgical grade silicone elastomer to seal the craniotomy. This technique involves thoroughly rinsing the chamber with saline and filling the base of the chamber with 3 mm or more of a 2-component liquid silicone elastomer. The elastomer comes in a syringe with 2 chambers and is injected through a sterilized tip, which is replaced for every application. To maintain the sterility of the contents of the syringe, we leave the sterilized tip on. Because the silicone remaining in the tip is sterile and hardens, it provides a solid sterile barrier between the contents of the syringe and the outside world. Each time the silicone seal is removed or replaced, the chamber will be thoroughly rinsed with saline and the walls will be dried. The cap is cleaned separately with hydrogen peroxide, a Betadine scrub solution and is then briefly sterilized and rinsed with sterile saline. When the silicone seal is to be left for more than 3 days, the seal will be checked 1-2 days after it is placed to make sure the seal is good. The presence of any liquid on top of the seal indicates a poor seal and will result in replacement of the silicone. Published work has shown that a well-sealed chamber can be left closed and untouched for at least 5-12 months (Spitler & Gothard, 2008). Only chambers that are hermatically sealed will be left for more than 2 weeks; in these cases, the seal and chamber will be checked at least once a week. Chambers that are not hermatically sealed may undergo standard cleaning (see next section) before the silicone is applied. This form of maintenance has several advantages over the standard cleaning regime. First, by reducing cleaning time and the application of hydrogen peroxide and betadine, it reduces the time that the animals are head fixed and not receiving rewards. Second, this technique not only keeps the dura sterile, but also significantly and substantially reduces granulation tissue and bone growth. As such, it greatly reduces the chance that a dural debriding surgery must be performed. Despite the exceptional cost of this protocol, it has many advantages to animal welfare, and we plan to use this technique.

(c) If a foul odor or purulent discharge is noted at the skin margin or within the well, we will alert DLAM veterinary staff.

2. Dural Debriding. Dural thickening is a common tissue response secondary to chronic exposure. Therefore, in order to facilitate the placement of electrodes, thinning of the dura will occasionally be done under general anesthesia: granulation tissue and regenerated periosteum will be removed using instruments such as curettes, Q-tips, dental probes and suction under a dissecting microscrope. Occasionally we are able to this when an animal is under anesthesia for an emergency repair, otherwise the animal is anesthetized with injectable, inhalant, or a combination of anesthesia as appropriate for the length of procedure and the individual animal as determined by the veterinary staff. In the past we have generally done 1 debriding per animal every few years and expect to do so at the same rate in the future.

3. Animal handling. The details of getting the animals into the primate chairs are described under the Physical Restraint section. The monkeys are trained to allow the experimenters to attach a pole to their collar. They then exit the cage, walk to a scale, sit on the scale, walk to a suitably sized primate chair and then hop into the chair. The chair, with the monkey in it, is then weighed to get an accurate weight measurement. Within the chair, they are restrained by the walls and neckplate and by attaching a chain, which is fixed to the chair, to the collar. They are then wheeled to the lab, where any routine maintenance of their implants is done, after which the chair is moved into the rig. During behavioral training, testing, cleaning and recording, the monkey's heads are affixed to a plate on the top of the primate chair.

4. Neurophysiological recordings. The activity of individual neurons, groups of neurons, or local field potentials in the brain will be studied in relation to the performance of the cognitive tasks to elucidate the underlying neural mechanisms. Neuronal activity will be measured in various regions of the brain by introducing microelectrodes through the dura using a previously implanted recording cylinder to which a multi-electrode microdrive will be attached. If necessary, guide tubes will be inserted through the dura to improve the reliability of the microelectrode recordings. Alternatively, bundles of microwires will be passed through the guidetube or inserted into the brain at the time of implant surgery or under a subsequent surgical procedure. A grid placed in the cylinder secures this stainless steel guidetube, and the guide tube may remain undisturbed in the brain for several months. In some cases, electrodes will be secured to the guide tube, detached from the electronic recording system, and left in the brain for up to several weeks. If more than momentary discomfort is involved, then 2% Bupivicaine w/o epinephrine will be applied to the dura. All guide tubes and electrodes will be sterilized before use as described above. Particular care will be taken to minimize neural damage during the placement of the guide tubes and electrodes since this has the potential to compromise the animal's sensorimotor coordination and hence jeopardize the entire study. Because the brain is not known to have nociceptors, intracerebral electrodes do not generally give rise to any pain or discomfort; such electrodes are well tolerated by human patients, and the implantation of the electrodes into the brain (as opposed to through the skull) are routinely performed without anesthesia.

5. Electrical stimulation. Stimulation through the recording electrode or through separately placed electrodes will be done to aid in the assessment of the functional organization of the recording region. Past experience indicates that this procedure is rarely stressful and will be done while the animal is awake since 1) correct placement of the electrodes can only be accomplished after first recording the neuronal activity associated with behavior, and 2) the oculomotor or other behavioral responses to the stimuli would be modified or even eliminated by anesthetics. Currents are usually limited to 100 uA or less, and are always injected bi-phasically. If the monkey responds to the stimulation in a manner suggestive of discomfort (e.g. grimacing, vocalization, squirming, etc.), then the electrical stimulation at that site will be immediately discontinued. Usually, microstimulation experiments are performed 3-5 days a week during the testing sessions. Within that session, between 25-50% of trials include microstimulation for a duration of 500 ms, although a range of durations from 50-1000 may be used. Generally, once the duration is set (say 500 ms) it will be used for all sessions within that experiment.

6. Chemical stimulation. An antagonist or agonist of the neurotransmitter gamma-amino-butyric acid (GABA), such as muscimol, will be injected via a Hamilton syringe to study their effects on the animal's behavior and on the response properties of the neurons thought to mediate that behavior. The quantities involved will be very small (microliters) and their effects are rarely stressful; the object is to investigate the physiological function of a very restricted region of the brain and it would defeat the purpose of the study if the injected substance were to spread to other regions. Injections will be done while the animal is awake since correct placement of the cannula/guide tube can only be accomplished after first recording the neuronal activity associated with behavior. If the monkey responds to the neurotransmitters in a manner suggestive of discomfort (e.g., grimacing, vocalization, squirming, etc.) then the animal will be anesthetized with ketamine, pentobarbital or gas anesthesia, and the enduring neural/behavioral effects studied after recovery from the anesthetic.

7. Somatosensory Stimulation. During the study of brain areas responsive to somatosensory as well as visual stimuli

the monkey's skin and underlying tissues will be stimulated with the sorts of stimuli that are routinely used in human clinical examination: puffs of air, wisps of cotton, or paper.

8. Anatomical Techniques. Magnetic resonance imaging (MRI) is a non-invasive method of obtaining high resolution images of the body. This method has proven particularly useful for imaging discrete structures in the brain. Researchers at the NIH have developed a method that combines stereotaxic localization with MRI imaging, which allows placement of guide tubes directed at given brain structures with a high degree of accuracy not previously possible. MRI can also provide us with an accurate image of the success of our interventions postoperatively. One example is the localization of the discrete damage resulting from injections of ibotenic acid into cortical and subcortical areas. Due to its non-invasive nature, MRI procedures are routinely utilized in human patients as a diagnostic tool to identify and study both normal and abnormal brain functions. To this end, we either utilize a nonferromagnetic sterotaxic headholder developed for this function or the Brainsight system. The Brainsight system is similar to the systems used in human neurosurgery to localize brain regions with MRI. With this system a frame that surrounds the coil is used to hold the animals' head stationary (using the previously implanted head post; if it's a new animal then we will have implanted either a head holding post or we will hold its head in place using blankets and other cushions on either side of its ears). In either case 5 removable fiducial markers are attached for image registration. The markers are visualized together with the head to provide registration of the brain image for subsequent surgical procedures and brain structure targeting under MRI guidance.
Sedated animals will be transported to the MRI unit using approved animal transportation procedures. This usually involves placement of the animal in a small, sealed transportation cage and then placement of the cage in a covered cart. Appropriate induction and maintenance anesthetic regime will be chosen by the veterinary staff from those given in the Surgery section of the protocol based on the individual animal and procedural needs. While in the MRI unit, the animal's oxygen saturation and heart rate will be monitored continuously with an MRI compatible pulse oximetry unit. In the event that this equipment fails, we will monitor the animals/vitals manually by taking breaks between scans every 15 mins. Documentation will occur during these intervals. A member of the veterinary staff will always be in attendance to monitor the animals' health during any MRI scans and provide anesthesia and any necessary emergency care. During the scanning session, the animal will be covered with blankets to maintain its body temperature. To aid in the imaging of target sites, some monkeys may be injected intravenously with Magnevist during the scanning session. To facilitate the localization of recording electrodes, MRI-imageable materials may be inserted through the recording grid directly, or through a plastic guide tube inserted in the grid. Examples of such material include quartz fibers, tungsten recording electrodes, or capillary pipettes filled with Vitamin E or Magnevist. Each session can last from 45 min to 2 hours, after which time the animal is transferred back to the home cage where it is monitored continuously until it awakens and is able to climb onto the perch, with records taken every 15 min. Animals tend to have MRIs prior to chamber implantation and again after implantation. Given that we do not tend to move or remove chambers often, this results in animals usually having two MRIs in a year and then usually having no MRIs for the following 2-3 years.

9. Common Behavioral Tasks.
i. Fixation tasks. In these tasks, the animal must fixate a central spot for the duration of the trial. Stimuli may appear in the periphery and the animal is rewarded for maintaining fixation in the center until the spot goes out.
ii. Visually and Memory Guided Saccade tasks. In these tasks, the animals must fixate a central spot for a few hundred milliseconds, after which a stimulus appears in the periphery. In the visually guided saccade task, the peripheral stimulus remains on and the animal must make a rapid eye movement (saccade) to it as soon as the central spot is extinguished. In the memory guided saccade task, the peripheral stimulus goes off and the animal must make a saccade to the remembered location as soon as the central spot is extinguished. In some versions of these tasks, other distracting stimuli may be presented away from the saccade target locations.
iii. Visual Search tasks. In center-out versions of visual search tasks, the animal fixates a central spot for a few hundred milliseconds, after which a circular array of stimuli appears. In saccade versions of the task, the animal must make an eye movement to the target as soon as the fixation point goes off. In manual versions of the task, the animal maintains fixation, but indicates the presence, absence or orientation of the target by pushing a button or releasing a bar. In foraging versions of visual search tasks, the animal fixates a spot for a few hundred milliseconds, after which an array of stimuli appears. In these versions of the task, the animal is free to look around the array until he finds the target. In these arrays, the stimuli are typically not arranged in a circle, but are spread out around the visual field.
iv. Change Blindness tasks. In these tasks, the animal begins a trial by fixating a central spot for a few hundred milliseconds, after which an array of stimuli appears for 500 ms, followed by a brief blank screen, followed by a reappearance of the array. In change trials, one of the stimuli changes and the animal must make a saccade to that stimulus. In non-change trials, the animal must maintain fixation.
v. Match-to-Sample tasks. In our main match-to-sample task, the animal is presented with a stimulus in a central location, where he is fixating, and two stimuli in the periphery. The animal must make an eye movement to the stimulus in the periphery that matches the stimulus in the central location. In this task, the timing of stimulus onset can vary greatly. In a delayed match-to-sample task, the animal is presented with a single stimulus (the sample), which is followed by a delay and then a second stimulus. If this stimulus matches the sample, then the animal must push a button or release a bar. In the same-different match-to-sample task, the animal is presented with a single stimulus (the sample), which is followed by a delay and then a second stimulus. If the stimulus matches the sample, then the animal must push one button (or release one bar). If the stimulus does not match the sample, then the animal must push a different button (or release a different bar).

10. Injection protocols.
i. IV injection: A syringe with a 20-25 gauge needle is inserted into a preexisting vascular catheter, or into a peripheral vein, such as the femoral vein, saphenous vein, or cephalic vein. The syringe plunger is briefly pulled back to ensure the needle is appropriately placed into the catheter or vein (ie a small amount of blood will be seen), then the injection is given over 10-30 seconds, or at an appropriate duration for the particular substance. The needle is withdrawn from the vein and placed in the sharps container. If the injection was made into a catheter, the catheter is flushed with saline. If the injection was made into a vein, the vein is held off for 30-60 seconds or until bleeding stops. A DLAM veterinarian will be notified if bleeding does not stop. IV injections are given only into anesthetized primates, so pain/distress is not expected.
ii. IM injection: A syringe with a 20-25 gauge needle is inserted into an appropriate muscle such as the quadriceps (cranial to the femur, or otherwise pointing away from the sciatic), the deltoids, triceps (needle pointing away from the bone), gluteals, or the epaxial muscles caudal to the kidneys. The plunger is briefly pulled back to ensure appropriate placement; no blood should be seen in the hub of the needle. Then the injection is made, the needle withdrawn slowly, and placed into a sharps container. IM injections are made either to anesthetized primates (no pain/distress expected), or to primates partially immobilized by use of a squeeze cage. If in a squeeze cage, the squeeze mechanism is released after the injection. Brief scratching at the area is normal. If unexpected signs are seen, a DLAM veterinarian is contacted.
iii. SC injection: A syringe with a 20-25 gauge needle is inserted into the subcutaneous fat, usually in the neck, back, or at the cranial junction between the rear limbs and the torso. The plunger is briefly pulled back to ensure

appropriate placement; no blood should be seen in the hub of the needle. Then the injection is made, the needle withdrawn slowly, and placed into a sharps container. SC injections are made either to anesthetized primates (no pain/distress expected), or to primates partially immobilized by use of a squeeze cage. If in a squeeze cage, the squeeze mechanism is released. Brief scratching at the area is normal. If unexpected signs are seen, a DLAM veterinarian is contacted.

2. List probable clinical responses to and potential complications of the nonsurgical procedure(s).

Local infection of implant from recording procedures.

Gas Anesthetic

NOTE: Gas anesthetics like isoflurane, halothane, enflurane, and ethane must be used safely. The Off ce of Environment, Health & Safety (EH&S) requires the use of a certified fume hood or a gas anesthet c machine that contains a scavenging dev ce (e.g., anesthet c gas machine w th charcoal filter; ducted fumehood or ducted b osafety cabinet; Crump WAG System; vaporizer w th a scavenging filter, such as F-air canister) when using gas anesthetics.

1. What gas anesthetic agent(s) will be used?

- ☐ Halothane
- ☑ Isoflurane
- ☐ Other:

2. Gas anesthetic(s) will be scavenged via:

- ☐ Certified Fume Hood:
- ☑ Other: Precision vaporizer with charcoal canister in the surgical suites in [redacted]

Scavenging Location

This section is empty.

Hazardous Agents

If you are planning to use rDNA, chemical or biohazardous agents (carcinogenic, teratogenic, or highly toxic substances; nanoparticles; human cell lines; or infectious agents) in live animals, you are required to provide the information about the agents below. The appropriate safety committee will review your request directly in the application.

Agent(s) that will be used:

Agent Name	Route of Administration	Volume	Time to Euthanasia	Approval Date
Formaldehyde	Perfusion through the heart	2 l	Perfus on occurs after euthanasia	
M dazolam	im	~1 ml	N/A	9/8/2014
Muscimol	Intra-cerebrally with Hamilton Syringe	less than 5ul	Animals are euth. at points discussed in other sections and not at a part cular point after inj.	8/1/2017

Prolonged Physical Restraint

See ARC Policy on Physical Restraint of Unanesthetized Animals. ARC policy defines prolonged physical restraint as restraint for longer than 15 minutes. It is NOT necessary to complete this section when the physical restraint is: (1) for brief restraint/examination, (e.g., for collection of samples or for injections), or (2) for an anesthetized animal. If devices such as restraint socks or squeeze cages are used, it is important that such devices be suitable in size and design for the animal being held. They must operate properly to minimize stress and avoid injury to the animal.

1. Rationale for Restraint:

Restraint of monkeys is essential to these experiments in order to: 1) protect delicate equipment and microelectrodes that are placed on or near a monkey's head during experiments for the purpose of monitoring neural activity; and 2) permit us to measure eye position so that we can present visual stimuli to retinotopically specific locations in the visual field repeatedly.

2. Describe the type of restraint device, dimensions, conditioning of the animal to restraint, etc.

During experiments, monkeys are seated in a custom-build plexiglass chair that is tailored to meet the size of the animal and to contain any excrement. The chair allows the monkey to move its limbs and body during an experiment to ensure continued comfort. We take care to ensure that there are no points of pressure between the monkey's body and the chair. In order to maintain and measure eye and head position, the monkey's head is held in place by connecting a surgically implanted head holding device to the top of the chair. We only restrain the head of a monkey during an experimental session or to facilitate medical treatment (care of implants, etc.). We do not transport monkeys during head restraint.
A monkey is gradually conditioned to restraint. For this process we usually water schedule the monkey, using water levels of approximately 40 ml/kg/day. First the animal is introduced to the pole. This usually involves leaving it sitting in the cage for a few days to a week. Once the monkey is acclimatized to having the pole around, the trainer will hook the pole onto the collar and then give the monkey food treats and water, while it is hooked. This is repeated several times a day. There are two approaches we use for this step. In one, the animal is hooked in the cage and, once it is used to being on the pole, it is taken out of the cage and lured onto a scale by means of food treats. Once the monkey is used to getting out of the cage and sitting on the scale, it is lured into the chair with food rewards and water. Then, as the animal becomes more confident, such treats are used to lure it into introducing the head into the chair's neck plate. The alternative approach is to use a special training chair with a vertical sliding door. The training chair is designed so that it directly abuts the cage and when the cage door is opened, the animal can only go into the chair. The animal is lured into the chair with food rewards and [redacted]

without being poled. Once in the chair, the sliding door can be closed and the chair moved away from the cage. Using this approach, the initial hooking and acclimatization to the pole occur while the animal is in the chair. Hooking the pole to the collar while the animal is in the cage occurs later in the process.
After the animal becomes obviously comfortable and confident in being chaired, the animal – well secured in the chair – is transported into the laboratory, where it is given copious treats and water ad libitum. Only when the animal clearly volunteers into the chair and has become acclimated to the lab and its rewards, does it have its head restrained for the first time. The animal's head is only restrained for a short time initially, and then as the monkey gets used to this restriction, the animal is introduced into the testing apparatus and habituated to progressively longer restraint and then testing sessions.

3. Restraint Duration and Frequency:

Monkeys sit in the chair for the duration of an experimental or training session (typically 2-4 hours, with a maximum of 6 hours). The end of the session is in large part dictated by the monkey. When the animal quits working, the experiment terminates and the monkey is returned to its home cage.

4. Describe how frequently the animals will be observed during the restraint period.
Please also describe criteria for removal of animals from restraint.

5. Will pain or discomfort be induced?

No

Species Restraint

Species	Number of Animals
Rhesus Monkey	8

Principal Investigator Assurance

After you have reviewed and answered yes to the items below, please click "Save" at the bottom of the page. Please note that the PI must complete this section. To determine your eligibility to serve as Principal Investigator of a research protocol, please refer to UCLA Policy 900 (Principal Investigator Eligibility) or contact the ARC administrative office (310-206-6308). If the terms of Policy 900 are not met, faculty sponsorship or principal investigatorship by a UCLA employee with faculty appointment may be required.

Regarding policies governing animal research at UCLA:

[redacted]

Yes	No	
◉	○	I agree to abide by all applicable federal, state, and local laws and regulat ons and UCLA pol cies and procedures.
◉	○	I am aware that deviat ons from an approved protocol or violations of applicable pol cies, gu delines, or laws could result in immediate suspension of the protocol.
◉	○	I understand that the attending veterinarian or his/her designee must be consulted in the planning of any research or procedural changes that may cause more than momentary or slight pain or distress to the animals.
◉	○	I declare that all experiments involving live animals will be performed under my supervis on or that of another qualified scientist. All listed personnel will be trained and certified in the proper humane methods of animal care and use prior to conducting experimentation.
◉	○	I understand that emergency veterinary care will be administered to animals showing evidence of discomfort, ailment or illness.
◉	○	I declare that the information provided in this appl cation is accurate to the best of my knowledge. If this project is funded by an extramural source, I certify that this appl cation accurately reflects all currently planned procedures involving animals described in the proposal to the funding agency.
◉	○	Any modificat ons to the protocol will be subm tted to and approved by the ARC prior to initiation of such changes.
◉	○	The experimental design has been refined in order to minimize the invasiveness of the proposed procedures.
◉	○	I assure that the proposed research does not unnecessarily duplicate previous experiments.

Agreement on electronic submission:

I understand that by submitting this document that this document will be sent to appropriate members for review. I further understand that once submitted for review, this protocol cannot be modified or changed unless so requested by the ARC. In addition, once approved, all changes or modifications must be subm tted for review and approval of the ARC prior to in tiation.

Completed by: [redacted] **9/4/2019**

FS Assurance

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