From: Russ Kick
To: records@ucla.edu
Subject: PRA request
Date: Monday, October 26, 2020 11:20:17 PM

Rise For Animals
333 Washington Street, Suite 850
Boston MA 02108

University of California, Los Angeles

Dear Public Records Officer:

This is a request under the California Public Records Act.

For the deceased primate known as #BH41, we request:

1) All intake records (including but not limited to acquisition forms, transportation forms, invoice, bill, paperwork with UW-Madison, intake medical exam, etc.)

2) The full IACUC-approved protocol that we was being used in

3) All notifications of adverse events, animal welfare concerns, noncompliances, etc. involving him

4) Daily care logs from January 1, 2020, until his death

5) All photos and videos ever taken that show him

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

Continuation Application

General Information	
Title:	[redacted]
Protocol #:	[redacted]
PI:	[redacted]
Status:	APPROVED_WITH_CODICIL
Approval Period:	5/13/2020-5/9/2021
Received Date:	4/30/2020
Type:	Continuation
Species:	15 Rhesus Monkey (Pain Category D)
Create Date:	4/1/2020 10:18:32 AM
Created By:	[redacted]
Owner:	[redacted]

Updated Sections
Animal Care
Contacts
Continuation Summary
Euthanasia Medications
Experimental Design
Hazardous Agents
Medications and Experimental Drugs
Non-Surgical Procedures
Number of Animals Used
Pain Category
Pain Category Assignments
Pain Literature Search
Personnel
Physical Restraint
PI Assurance
Pre-Review
Response to Pre-Review
Scavenging Location
Species Restraint
Species Surgery
Surgery
Tissue Collection

Personnel Certifications Due:

[redacted]
- MHQ (valid until 8/8/2020)

[redacted]
- Aseptic Surgery

[redacted]
- MHQ (valid until 7/5/2020)

[redacted]
- Aseptic Surgery

[redacted]
- MHQ (valid until 8/1/2020)

Notes:

- General Certification Test: Offered through CITI program (**http://www.citiprogram.org**). Please ensure your affiliation is listed as UCLA and complete the Animal Research Basic Course.
- Medical History Questionnaire (MHQ): Offered by the Occupational Health Facility (**http://mhq.healthsciences.ucla.edu/**).
- Species Specific Training: Please visit the DLAM website: **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 participate in survival surgery procedures until [redacted] has completed the DLAM in person aseptic surgery training (Note: DLAM is not offering training at this time).

Continuation Summary

Please provide the appropriate information regarding changes to this protocol. Then update the respective sections.

1. **Will you be planning on making changes to this protocol? If you answer "yes" to this question, please address Questions 2 and 3 and update the appropriate sections that are affected by these changes. Please note that you are not required to update every section of your protocol.**

(●) Yes ( ) No

2. **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

3. Indicate if you will be making any Significant changes to the following:

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.

General reorganization of the text was included to match my other 2 protocols involving similar procedures and the same species.

Based on our experience with one animal this past year, we modified our Simíniscope mount design and our surgical procedures to remove the coverslip approach and to add a cap/helmut that encloses the entire mount implant for protection. These changes are documented in the appropriate sections.

Typos throughout were corrected.

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 change in experimental procedures.

Based on our experience with one animal this past year, we modified our Simíniscope mount design and our surgical procedures to remove the coverslip approach and to add a cap/helmut that encloses the entire mount implant for protection. These changes are documented in the appropriate sections.

We expect greater success by removing the glass cover slip approach and adding a cap to enclose the entire implant.

4. In order to assist reviewers, briefly describe in lay terms the changes you are making and complete the appropriate sections.

General reorganization of the text was included to match my other 2 protocols involving similar procedures and the same species.

Based on our experience with one animal this past year, we modified our Simíniscope mount design and our surgical procedures to remove the coverslip approach and to add a cap that encloses the entire mount implant for protection. These changes are documented in the appropriate sections.

Typos throughout were corrected.

5. To assist the ARC in documenting scientific progress arising from use of animals under this protocol, please provide ONE of the following:

- ○ Citation(s) of presentations or articles resulting from this protocol (either accepted or submitted). Please include an abstract.
- ◉ A brief (1-2 sentence) update regarding progress made toward achieving the scientific objective(s) of this protocol.
- ○ A copy of the most recent annual progress report submitted to the funding agency.

If the scientific progress documentation is in a text format, please paste (or type) it here. Otherwise, you will need to submit it to the ARC as a hard copy.

We performed surgical procedures as outlined in the protocol on 1 animal and assessed performance of the GRIN lens implant and the V.3 of the Siminiscope design. As a result of this procedure and outcome, we made considerable modifications in the design of the implant and its installation. We also designed a new cover to protect the entire implant.

6. Please indicate whether any adverse effects or unanticipated problems have been experienced, including higher than anticipated mortality/morbidity regardless of the cause. If so, please provide an explanation of how these events/problems were resolved.

Yes

We performed two major surgeries on this animal as per our protocol, for the development of the new miniscope implant procedure and device. The results from these procedures were extremely informative - we developed new ideas for redesigning the mount and its protective device to ensure its stability, viability and safety for the monkey. As a result of this one set of experiments in this one animal, we are on version 3 of our implant design (in collaboration with our engineer colleagues) and are well on our way to having this methodology work. We are eager to test our new design in a new monkey. Our goal is to have an implanted device that remains viable, stable and infection free for at least 2-6 months. The long term goal is years.

This animal was euthanized because no additional data were needed from it. However, during a routine MRI, I noted an incidental finding suggesting a sub-acute hematoma at the site of one of our 3 injections. I asked the vet if we could provide treatment for this to ensure the animal remained comfortable until its planned euthanasia. That treatment was successful, the animal was healthy and showed no neurological signs at the time of euthanasia (nor at the time of MRI).

Our next planned surgery will not change in procedure based on the event described above as we will be targeting the two areas that were not associated with the hematoma. I will also use the MRIs from the previous and new animals to determine whether our angle of penetration needs to be altered. The histology will be used to confirm (or negate) the MRI results of a sub-acute (and subclinical) hematoma.

7. Please respond to the following questions regarding alternatives to the use of animals. If you answer YES to any of these items, please explain.

A. Have any alternatives become available since the previous ARC approval that could replace the use of animals to achieve your research and/or teaching goals?

No

B. In order to reduce potential pain/distress, have any procedural refinements been made since the previous ARC approval?

Yes

I am currently in discussion with an engineer to design and develop a new chair and chair training procedure that would eliminate the need for pole and collar restraint and training.
https://www.ncbi.nlm.nih.gov/pubmed/30738106

C. Has the number of animals required for the study been reduced since the previous ARC approval?

No

Number of Animals Used

List the species and the total number of animals used in the previous year.

Species	Total Number Used
Rhesus Monkey	1

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 institution please submit the most recent approval notice 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 with the ARC, you must submit an Application 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:**	Ph.D.
Fax:		**Dept:**	[redacted]
Status:	Faculty		

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?

Yes

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 been working with monkeys since [redacted] and has been performing cranial and ophthalmic surgeries as well as behavioral and cognitive assessments and experiments and electrophysiological experiments in monkeys since [redacted] [redacted] is fully trained in all aspects of all procedures proposed and will be responsible for all work, oversight and compliance of the proposed work as well as training of others on this project, as it pertains to the use of monkeys.

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

Dr [redacted] will perform all aspects of this protocol from surgical procedures to behavioral, cognitive assessments and measurements of optical signals from the Siminiscope. [redacted] will oversee all aspects of the protocol and ensure the proficiency and competency of all personnel involved in the project.

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

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

What role will this person be performing in this protocol?

Co-Investigator

Which species will this person handle in this protocol?

Will this person handle animal tissue in this protocol?

No

Will this person be involved with Survival Surgery Procedures?

No

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.

Dr. [redacted] is a faculty member at UCLA and key personnel on this project. [redacted] will work together with Dr. [redacted] and [redacted] to develop and implement the miniscope for use in monkeys. Dr [redacted] will be present in the surgery suite but will not perform procedures nor handle animals. [redacted] will participate as required for testing of the miniscope together with [redacted] but will not handle or perform any procedures on monkeys. [redacted] will train [redacted] as needed.

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

Key personnel on the [redacted] award and PI on other awards that led to the development of this technology for mice here at UCLA. Will collaborate with [redacted] and [redacted] on the development and implementation of the miniscope for monkeys.

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted]. View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	M.D., Ph.D.
Fax:		**Dept:**	[redacted]
Status:	Faculty		

What role will this person be performing in this protocol?

Co-Investigator

Which species will this person handle in this protocol?

Will this person handle animal tissue in this protocol?

No

Will this person be involved with Survival Surgery Procedures?

No

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.

Dr [redacted] is a faculty member at UCLA and CO-I on this project. [redacted] will work together with Dr [redacted] an [redacted] to develop and implement the miniscope for use in monkeys. Dr. [redacted] will be present in the surgery suite but will not perform procedures nor handle animals [redacted] will participate as required for testing of the miniscope together with [redacted] but will not handle or perform any procedures on monkeys. [redacted] will train [redacted] as needed.

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

CO-I on th [redacted] award and PI on other awards that led to the development of this technology for mice here at UCLA. Will collaborate with [redacted] and [redacted] on the development and implementation of the miniscope for monkeys.

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

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

What role will this person be performing in this protocol?

Co-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?

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.

Dr [redacted] attained a B.S. in biomedical engineering fro [redacted] in [redacted]. After receiving [redacted] Ph.D. in Neuroscience from [redacted] in [redacted], [redacted] conducted postdoctoral work at th [redacted], [redacted] Dr. [redacted] arrived at the [redacted] in [redacted] to establish [redacted] own research laboratory and to head a core facility for brain imaging in nonhuman primates [redacted] is now a [redacted] [redacted] is also the

[redacted], a joint core facility sponsored by the [redacted]

Dr[redacted] has been performing monkey behavioral imaging electrophysiological and surgical procedures since[redacted]

In Ma[redacted], the ARC have approved training exemptions as per ARC Policy for Visiting Scientist."

MHQ Waiver has been submitted February[redacted], see amendment[redacted]. ARC Office[redacted]

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

Dr [redacted] will collaborate with D [redacted] on neurosurgical techniques and will participate in surgeries.

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

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

What role will this person be performing in this protocol?

Co-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?

Yes

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.

In Ma[redacted] the ARC have approved training exemptions as per ARC Policy for Visiting Scientist.

Dr [redacted] is a [redacted] has been working with NHP since[redacted] and is an expert in monkey neurophysiology.

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

Dr [redacted] will be collaborating with Dr. [redacted] on neurosurgical techniques and will participate in the surgeries.

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

Email:	[redacted]	UID:	[redacted]
Phone:	[redacted]	Degree:	M.D., Ph.D.
Fax:		Dept:	[redacted]
Status:	Student		

What role will this person be performing in this protocol?

Co-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?

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.

Dr. [redacted] is [redacted] at UCLA, who received [redacted] MD in [redacted] and [redacted] PhD in [redacted]. [redacted] did a surgical residency from [redacted] [redacted] is board certified in [redacted] and the [redacted] since [redacted] [redacted] specializes inn nthe surgical treatment of movement disorders including PD and peripheral nerve disorders as well as radiosurgery. [redacted] has experience performing neurosurgical procedures in humans and monkeys.

In May [redacted], the ARC have approved training exemptions as per ARC Policy for Visiting Scientist.

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

Dr. [redacted] will perform surgeries together wit [redacted].

Will this person handle radioactive materials or radioactive animals?

No

Co-Investigator

[redacted] View Person Detail

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

What role will this person be performing in this protocol?

Co-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?

Yes

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.

In Ma [redacted] the ARC have approved training exemptions as per ARC Policy for Visiting Scientist."

Dr [redacted] is [redacted] is a primate neurosurgeon with over [redacted] years of experience.

MHQ Waiver has been submitted March [redacted], see amendmen [redacted] ARC Office [redacted]

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

Dr [redacted] will collaborate with Dr. [redacted] on neurosurgical techniques and will participate in surgeries.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

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

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] received [redacted] PhD from [redacted] working at th [redacted] in [redacted]. [redacted] was a post doctoral fellow at [redacted] fro [redacted] Dr [redacted] has expertise in behavioral training, cognitive assessment, pole and collar and chair training procedures in rhesus monkeys. [redacted] is also skilled at clinical procedures including IV placement, femoral and saphenous blood collection, nasogastric gavage and drug administration including IM IV and SQ routes. Dr [redacted] has has experience with intracranial injections and the use of

chemogenic neuroscientific approaches including DREADDS in monkeys. For procedures specific to the [redacted] lab, Dr. [redacted] will be trained in all aspects and overseen by the PI until proficient.

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

Dr [redacted] will participate in all aspects of the protocol including behavioral training and experimentation as well as electrophysiological experiments and surgical procedures. [redacted] will not perform any surgical procedures until trained and assessed for proficiency by the PI. The same for all other aspects of the research performed.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

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

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.

[redacted] has more than [redacted] years of academic animal research experience and in this time has worked with Mice, Rats, Rabbits, Pigs and Sheep (Mice and rats from [redacted] an [redacted], rabbits, pigs and sheep from [redacted]. In this time, [redacted] has developed surgical and anaesthesia skills and actively engaged in new anaesthesia techniques and advancing surgical procedures. [redacted] has Excellent working knowledge of animal technologies relevant to academic research.

Examples of [redacted] specific experience include:
• involved in more than 800 large animal surgical procedures.
• Sedated and anaesthetised more than 800 large animals using gaseous and intravenous anaesthetic agents.
• Trained in drug dosing and administration and fluid management.
• Assisted in the setup and maintenance of more than 200 cardiopulmonary bypass procedures and more than 150 Cath Lab procedures in pigs
• Assisted in the recovery of a similar number of animals where I have had to provide effective post-operative pain assessment plans to administer antibiotics and analgesics.

[redacted] is also trained to make independent, informed decisions on administration of drugs required in different surgical situations. [redacted] has taken all the required training classes from DLAM and is currently undergoing proficiency assessments by Dr. [redacted].

Regarding specific experience with monkeys, behavior and assessment, [redacted] will be trained and overseen by the PI and the PI has arranged for [redacted] and all other members of th [redacted] lab to attend a two day training workshop a [redacted] primate center on training and assessment of laboratory rhesus monkeys.

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

[redacted] will participate in all aspects of the procedures including surgical procures as described in this protocol. [redacted] will oversee all aspects of care and compliance of our procedures and will carry out medical treatments as prescribed by the veterinarians.

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?

Non-Human Primate (other)

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] received her ALAT i [redacted] and [redacted] RVTg i [redacted]. [redacted] has experience with species as diverse as mice rats, frogs, monkeys, and tree shrews since [redacted] when [redacted] receive [redacted] Bachelor degree in Animal Health Science. during [redacted] served as a veterinary technician where [redacted] supported the care and treatment of companion animals. Sinc [redacted] worked a [redacted] as an Animal Care technician supporting rodent and non human primate research programs. [redacted] skill set includes, husbandry, monitoring and treatment of animals under fluid and food regulation, anesthesia monitoring, IM PP and SQ administration of medications to mice and monkeys [redacted] also has experience handling monkeys with the pole and collar method as well as training monkeys for arm presentations for blood draws and training in closed door method of chair training [redacted] has extensive experience with mouse colony management as well as genotyping and heath assessment and medication delivery. [redacted] has taken all the required DLAM training classes including the species specific training for monkeys. [redacted] will be taking the aseptic training class in person once it is offered. And [redacted] will not participate in surgical procedures until that is complete.

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

[redacted] will be involved in all aspects of the procedures outlined in this protocol. A key responsibility will be to assist the clinical care manager and to the PI to oversee clinical and research operations in the lab. [redacted] will train monkeys, clean monkeys, participate in surgical pre peri and post procedures and monitoring once [redacted] has completed the aseptic surgery training class.

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?

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.

[redacted] is a laboratory assistant II, who graduated i [redacted] with a B.S. in Neurobiology, Physiology and Behavior from [redacted] has been working with monkeys in the [redacted] lab sinc [redacted] and is proficient in all aspects of handling and electrophysiology and behavioral experiments with monkeys independently. [redacted] will be overseen by [redacted] or [redacted] designee.

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

[redacted] will assist the PI or [redacted] designee in handling animals, daily maintenance, and experimentation. [redacted] will also perform behavioral and electrophysiological experiments. [redacted] will not perform surgical procedures but may assist and has been trained and will be overseen by the PI or [redacted] designee on all aspects of the research and experimental procedures.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

Email:	[redacted]	**UID:**	[redacted]
Phone:	[redacted]	**Degree:**	M.D.
Fax:		**Dept:**	[redacted]

Status: Post-doctoral/Research Fellow

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.

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 [redacted] 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.

Dr. [redacted] will be providing colony oversight when deemed necessary, these times may include when the PI and the lab manager are unavailable. [redacted] may handle the animals (if needed) and perform non-surgical or surgical procedures in this protocol if PI is unavailable to do so (in an emergency situation only).

Will this person handle radioactive materials or radioactive animals?

No

Personnel

[redacted] View Person Detail

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

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.

[redacted] is a first year PhD student in the [redacted]. As an undergraduate volunteer in a monkey lab at [redacted] from [redacted] worked on the design and programming of a memory task for monkeys. This included sitting in on recording sessions with monkeys to make sure the task was running as expecte [redacted] also performed analysis of data obtained from monkeys. [redacted] also has experience with mice and rats [redacted] wher [redacted] developed an episodic memory task for mice and wher [redacted] performed experiments designed to study sleep [redacted] has handled mice and rats but has never handled monkeys directly. As such I will ensure tha [redacted] is adequately trained and supervised and I will ensure [redacted] competence and proficiency before being allowed to handle monkeys independently.

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

[redacted] will handle and train monkeys to entire a primate chair and perform a decision making task [redacted] will be trained and overseen until proficient by [redacted] and/or [redacted] designee. Once proficient [redacted] will also be trained to ensure the care of the animals such as cleaning of chamber implants and perform electrophysiological and behavioral experiments as outlined in the protocol.

Will this person handle radioactive materials or radioactive animals?

No

Personnel

DLAM Staff View Person Detail

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

DLAM personnel may participate in anesthesia induction and maintenance and monitoring as needed for the performance of procures and surgeries as outlined in this protocol. They are trained and overseen by DLAM veterinarians.

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

DLAM personnel may participate in anesthesia induction and maintenance and monitoring as needed for the performance of procures and surgeries as outlined in this protocol. They are trained and overseen by DLAM veterinarians.

Will this person handle radioactive materials or radioactive animals?

No

Contacts

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

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:

NIH - NATIONAL INSTITUTES OF HEALTH

Agency Code:

001775

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.):

The proposal indicates that some preliminary testing will be performed in mice. That work will be performed as part of the current mouse protocols from the [redacted] lab (protocol number [redacted]). This current protocol request is to cover the monkey work performed here at UCLA only.

Rationale

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

Our goal is to develop an open-source wireless miniature microscope to monitor the activity of multiple brain cells simultaneously in alert monkeys. Although mice are an excellent model for many circuit-based neuroscientific questions, the key to developing a detailed understanding of how brain circuits function in humans will be to perform circuit-based experiments in an animal model that more closely resembles humans, both in brain and behavioral sophistication. Our goal in this application is significant because we propose to fill this important gap in technology allowing for the first time, imaging experiments in alert and ultimately in freely moving monkeys.

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

The economic burden of neurological and neuropsychiatric disease in the US exceeds that of cancer and heart disease combined. It is therefore, imperative that we develop novel tools to begin understanding the complex neuronal circuits that give rise to our sophisticated behavioral and cognitive abilities that are often affected in neurological and neuropsychiatric diseases.

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.**

Our goal is to image multiple neurons at the same time, ultimately during performance of complex cognitive tasks in freely moving monkeys. Species lower than monkeys are incapable of performing such tasks in large part because they lack the regions of the brain (eg., visual areas V4 and IT, pre-frontal cortex) and their associated connections, that are specialized for these processes in higher mammals.

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 propose to develop the first, wireless miniaturized microscope for measuring brain signals in alert monkeys. We will make the technology available to other scientists across the world who are interested in using it in their laboratories. This technology will be optimized for use in regions of the brain close to the surface of the skull as well as regions of the brain located deeper.

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.**

OVERVIEW OF PROCEDURES FOR ALL EXPERIMENTS

The following procedures are required for all experiments performed in the laboratory under this protocol (more detail is provided in other appropriate sections). When we prepare an animal for an experiment (my definition of experiment is a recording or manipulation of brain activity), the ideal scenario is:

1. The animal is first trained (using only positive reinforcement methods - see SOP 2 Chair Training Procedures) to get into the chair willingly and cooperatively and be comfortable staying in the chair for extended periods (usually ~5 hours, always <12). Dr. [redacted] is currently working with an engineer to design new chairs that will allow for training of monkeys to jump directly from their home cages into the chair that then can be wheeled into the lab for experiments. This new method will mean that we no longer have to pole and collar train our animals, which is considered to be a significant refinement. Please see https://www.ncbi.nlm.nih.gov/pubmed/30738106

2. Next, they are implanted with a fiducial marker for accurate MRI imaging. They receive an MRI to provide us with information to perform subsequent surgery under MRI guidance. This method of MRI guided neurosurgery is virtually identical to what is used in humans (the hardware and software we use was adapted from that used in the human neurosurgical theater). This method of MRI guided neurosurgery is a significant refinement because, when combined with a contrast agent, we can also map out large blood vessels and install our chambers in a way that minimizes (but does not mitigate) chances of intracranial bleeding during subsequent experiments involving electrode penetrations into the brain.

To ensure accurate MRI guided surgery, we rely on fiducial markers that are placed close to the skull during the imaging procedure. In one method, we use a custom designed mouth bar that holds the fiducial markers, but we have found that this method is unreliable as it is difficult to recapitulate the precise position of the mouth bar during surgery. The alternative approach is to implant a small (5-10mm diameter) head holder onto which the fiducials can be attached during the scan. This requires attachment to the skull with 3-4 ceramic screws and can be replaced by the head holder required for subsequent behavioral and electrophysiological experiments.

Animals will undergo MRI scanning, at least once before surgery and 1-4 times after surgery used for targeting/assessment of mount and implant integrity. As needed, and as done routinely in humans, we may use contrast agents, such as MION, gadolinium and feraheme (described in non-surgical procedures). Further detail is also provided in non-surgical procedures.

5. MRI surgical planning is performed next, in which we design a skull implant that will include a head holding device as well as up to 3 craniotomies that will served as conduits to the brain for injections of Ca++ imaging dyes and implantation of GRIN lenses. At this stage a skull helmet is also designed to protect the implant of the GRIN lenses (and Siminiscope described later).

6. All monkeys will undergo up to three craniotomy surgical procedures. A small (1-10mm diameter) craniotomy is performed and AAV1-Syn-GCAMP6f (or new GCamp as the technology evolves) will be injected at multiple local sites into the brain region of interest (we will start with V4 / LIP and DLPFC and when surgical protocols are completed, we will target the deeper SC and striatal sites). Next, the GRIN lens and the mount that holds the GRIN lens will be implanted close to the craniotomy. These devices will be secured to an acrylic pedestal mounted to the skull through the implantation of 10-20 ceramic skull screws. The entire device will be sealed with a removable cap (helmet) for protection and safety.

After at least 4-6 weeks recovery time, monkeys will be chaired and the removable, wireless mini-microscope will be secured to the GRIN lens for initial testing. This procedure is equivalent to our current procedures for electrophysiologcal recordings except it is non-invasive, the scope will be attached to the implant and not enter the brain. Current procedures as approved in protocol [redacted] in which we mount an electrode holder on the implanted chamber.

The Siminiscope will be sterilized using EtOH Gas at the time of surgery. But note that the surgical procedure proposed is very similar to what we currently use to implant chambers and that the scope will sit on top of the mount embedded in the chamber but will be not permanently affixed. It will not come into contact with any part of the monkey brain or tissue as it will slide over the glass coverslip embedded in the chamber. This is all sealed so does not required cleaning or other maintenance.

7. Ca++ Imaging measures are performed next. Monkeys will sit in a primate chair and perform behavioral tasks (video games - see non surgical procedures) while the Siminiscope is placed on the implanted lens (completed enclosed and no tissue is exposed so does not require sterility or anesthesia) and images are acquired. This will be performed over a time period of at least one year or less depending upon the integrity of the lens (this is one of the variables we are interested in learning in this project - are these GRIN lenses and scopes viable in monkeys and how long can imaging occur once implanted?)

8. At the end of imaging (1 month - 1 year or less if the imaging integrity declines), all animals will be euthanized by thoracotomy and exsanguanation.

Our experimental endpoints are determined by the trainability of the animals, whether or not they are still willing and able to perform behavioral tasks, the success of the GRIN lens implant and imaging procedure, or if a veterinarian deems an animal unfit for experiments.

9. All animals may require fluid management to ensure motivation for behavioral training. This training includes pole and chair training as well as training on behavioral tasks to be performed in a chair during imaging procedures. All attempts are made to have monkeys perform tasks without fluid management but in our experience, more difficult learning requires more motivation and thus fluid management is the safest, most effective procedure to provide motivation and positive reinforcement in the form of desired fluids (eg., apple juice, sugar free koolaide). Further described in non-surgical procedures. Water management SOP provided.

10. For all procedures animals must be monitored closely for the first 48hrs post operatively, a minimum of two checks per day must be performed. Minor procedures will be monitored for up to 10 days total which can be reduced or increased on a case by case basis through discussion with the veterinarian, and is dependent on how well the monkey is doing. For major surgical procedures (e.g. craniotomy) there will be a minimum of a 10 day post op monitoring period in which the animals are monitored at least twice daily. All post operative monitoring will be done by DLAM staff and/or laboratory staff and records of the animal, its surgical site and medications received, are recorded.

NUMBERS OF ANIMALS
We are requesting 15 animals to develop and test the mini-microscope (Siminscope - pronounced like Jiminy). To continue this project and build upon what we learned this past year, 15 animals will receive injections of of AAV1/2 Syn GCamp6f(s) into the SC and/or V4 and/or LIP and/or DLPFC and/or the striatum. The precise number of animals required for the testing of different areas will be determined as the development of the technology evolves over the course of the three year grant award. We will start with 3 animals for V4/LIP/DLPFC testing and and 12 animals for SC, LIP and striatal testing. We expect to require more animals for the SC, LIP and striatal experiments, since these areas are deeper and we will simultaneously be developing surgical protocols for GRIN lens implantation (discussed in the Surgery section). We expect to collect a minimum of 100-1000 neurons per imaging session per animal. We also expect to perform multiple imaging sessions for at least 1 month to 1 year or until the lens is no longer able to create images (no longer than 3 years). The data collected will be used iteratively to determine what aspects of the Simini-microscope and mount system require additional developments and improvements. We will define success as the ability to collect this quantity of data multiple times over the course of at least one month.
This is a protocol designed to develop new surgical procedures and translating imaging technologies developed in mice to monkeys. We will try surgical approaches and design implants and assess their effectiveness and how long they last by making Ca++ images. If we are able to acquire at least 2 months of imaging from a single monkey and the implant integrity remains sound, we will consider our goal accomplished. We can only perform up to 3 craniotomies on any given animal. Therefore, we need multiple animals to trial the new designs.

All procedures requiring administration of drugs to monkeys will use pharmaceutical drugs. Those that cannot be such as the AAV GCamp, will be filtered through a 0.22um filter prior to use provided this does not reduce the viral titer.

Timeline/sequence of events animals will experience:

1) Chair training including fluid management as needed.
2) Placement of fiducial marker for MRI (one surgery).
3) Minimum 5-7 days recovery from above surgical procedure.

4) MRI scan.
5) Minimum 5-7 days recovery from MRI.
6) Surgical planning.
7) Installation of head holder, craniotomy, AAV/gCamp injection, implantation of GRIN lens and mount and cap.
8) Minimum 4-6 week recovery period to ensure osteointegration of implant.
9) Begin behavioral and imaging assessments/measurements.
10) Additional surgery (craniotomy) as needed to add GRIN lens and mount (not to exceed 3)
11) Minimum 5-7 days recovery.
12) Begin behavioral and imaging assessments/measurement.
13) End experiments when statistically reliable data are acquired or when lens stops giving images, whichever comes first.
14) Euthanasia and tissue harvesting.

Our animals are usually purchased from the [redacted] in [redacted] or [redacted]. Our preference is the [redacted]

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 which that procedure is applied. Examples of potentially painful/distressful procedures include, but are not limited 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 conscious 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 significant 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 with the experimental design (Examples: pain research; toxicity testing.)

Species:	Rhesus Monkey
Strain or Breed (if applicable):	maccaca mulatta
Average Weight:	4-15Kgs
Sex:	Mixed
Pain Category:	D
Previous Number of Animals Approved:	15
Change in Number of Animals Needed (+/-):	0
Number of Animals Needed for the 3 Year Period:	15

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.

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

☑ Other: We contact the vets at any signs of inappetence and weight loss >10%

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 anesthetics, unless withholding such agents is justified for scientific reasons and will continue for only the necessary period 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:

In order to develop other model systems we need to understand the functioning of the intact brain in an intact organism (animal), which is the goal of our research. Non-invasive methods for measuring brain activity at the spatial and temporal resolution required for our experiments at the depth in the brain that we study do not yet exist. Furthermore, the behaviors we study (eye movements and higher mental function) do not appear the same in lower species as humans. Mice and rats for example, either don't have or have considerably underdeveloped areas of the brain that we study in monkeys (eg., ventral visual cortex and prefrontal cortical areas).

Our new grant award is designed specifically to develop this imaging technology in monkeys to fill a gap in knowledge since this technology currently works only in rats and mice.

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

We have many mechanisms and refinements in place to minimize potential pain and stress in our animals. In fact, pain, stress or distress would be counterproductive for our experiments since we need the animals to be comfortable and healthy to perform complex behaviors.

1) All surgeries are done by experienced surgeons.

2) All surgeries are scheduled in advance with DLAM veterinarians.

3) All surgeries are performed with trained personnel in attendance, to provide anesthesia and scrub nursing support. Training is signed off on by DLAM veterinarians.

4) All surgeries are performed using aseptic (sterile) techniques.

5) All surgeries are done by fully qualified and trained personnel in anesthesia and appropriate post operative analgesics are provided to eliminate pain.

6) All surgeries are performed with pre- and post- operative antibiotic treatment to minimize the chances of infection development post surgically.

7) We use the Brainsight neuronavigation system to perform MRI guided surgeries including visualization of the brain targets and vasculature (to some extent) allowing for surgical planning that optimizes success and minimizes possible bleeding events.

8) We follow well-established procedures, vetted by veterinarians and human neurosurgeons, for handling and treating brain trauma that may result from introducing devices (used for injections or physiology) into the brain. These procedures are outlined in the animal are section. Veterinarians are contacted immediately for further consultation whenever negative events are suspected.

9) We are not planning to use eye loops as the precision of the magnetic induction means this technique is no longer needed. As such we do not expect any issues associated with this procedure. None of our current monkeys have eye loops.

10) [redacted] recruited four additional surgeons to collaborate on developing surgical protocols for these experiments.

THE PI HAS ACCESS TO THE LATEST METHODS

The PI is in regular contact with scientists and veterinary colleagues across the country and we have regular discussions on new/different methods for all procedures. The PI serves as editor for neuroscientific journals and subscribes to Lab Animal to keep up to date on any novel procedures that can be implemented to refine our procedures. For example, DLAM veterinarians recently suggested a potential refinement in water control procedures used for pole and collar and chair training of our animals. We are implementing the non invasive eye tracking method for these proposed experiments. Finally we are implementing a new chair design system that would remove the need for pole and collar training and restraint (see below).

PROCEDURES ARE OPTIMIZED FOR ANIMAL WELL-BEING

An understanding of brain function in normal and diseased states requires the use of techniques that are too invasive to perform in healthy humans. These experiments must involve subjects who are awake and actively engaged in the behavioral tasks. Many of these tasks involve skilled visuomotor and cognitive behaviors that are roughly analogous to performance of a video game. Sensory stimuli are presented and behavioral output, such as eye movements or touch screen choices, are measured precisely to understand normal brain function as well as how brain systems go awry in diseased states. Non-animal alternatives for these procedures are not available at present and lower animals cannot perform many of the them. The behavioral measures we use are foraging opportunities, and due to the cognitively demanding nature, are a source of psychological enrichment [redacted]. Note also that the electrophysiolgical methods we use - that of inserting electrodes into the brain to monitor active in alert animals - is performed routinely in the context of human functional neurosurgery in which the human needs to be alert to respond to the surgeon's queries as they assess brain regions controlling, for example, language and movement.

FLUID MANAGEMENT IS SAFE AND OPTIMIZED FOR ANIMAL WELL-BEING

We use positive reinforcement and preferred fluid for training and handling our animals. Management of access to the reinforcer is required to ensure animals are motivated for the reinforcer and in our experience, animals that are not motivated simply do not work well or work for only a very few trials. Fluid management can maintain animals in a normal physiological and healthy state if performed carefully, as monkeys are able to regulate their osmolality levels tightly within normal limits within the context of our water management protocols [redacted]. In some cases we have seen monkeys work for a few trials for highly preferred fluid (Tang, KoolAid), without managing access to other fluids. However, to collect statistically reliable data, 1500-3000 trials per experimental day are required. For this, regardless of the type of fluid provided, animals need to be motivated. We can use food reward, but generally the number of trials obtained with this method is insufficient for rigorous statistical analysis. Moreover, the chewing creates an artifact that interferes with our electrophysiological recordings. Evidence indicates that the water management procedures we use maintain animals in a physiologically healthy state and are similar to what would be experienced by these animals in the wild, in which, they have to forage to obtain their daily rations of flui [redacted]. Evidence also indicates that animals prefer to work for fluid than for other socially stimulating reinforcers [redacted] Furthermore, animals, like people, must be motivated to perform tasks that they may initially find difficult or about which they are uncertain. However, once trained well, monkeys often appear to enjoy coming to the lab (they cooperate with the handler and present their collar for removal from the home cage when trained properly). We view the time in the lab as psychological enrichment in which the animals have an opportunity to play video games as a foraging opportunity.

ANESTHETICS AND MEDICATIONS

Whenever new anesthetics or analgesics are used in laboratory animal veterinary practice,

we are more than happy to implement these as per veterinary suggestions. Likewise for other procedures we use.

RECENT UPDATES

We are currently working with an engineer to design new chairs for use with our monkeys. If successful these chairs will allow us to forgo training with the pole and collar method as animals can be trained to jump directly into the chair from their home cages. This would be a significant refinement and also considerably reduce our time to experiments. [redacted]

I recently recruited two new personnel to our team. We will be working together to train our monkeys to present a limb for blood draws thus minimizing the number of sedations animals may have to experience. The procedures for training are based on positive reinforcement methods (as we only use for all training) and will proceed in step wise fashion from sitting at the front of the cage to limb presentation.

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

We will use a minimal number of animals to ensure adequate testing of the new Siminscope. Our metric of success will be evidence of robust Ca++ signals that appear in response to events within a behavioral task, from area V4 / LIP/ DLPFC of cortex and the SC in the brainstem and the striatum (visual stimuli, eye movements). In total, a maximum of 15 animals will be used for continuation over the 3 years of the NIH award and protocol.

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 Regulations §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-mentioned regulatory requirement and in accordance with UCLA's Animal Welfare Assurance on file with the National Institutes of Health Office 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 provided a written narrative description of the methods and sources used to determine alternatives were not available." Please also note that the Committee recommends the use of keywords that are specific 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
- [x] Animal Welfare Information Center
- [ ] BIOSIS
- [ ] Current Contents
- [x] Other:

Dr. [redacted] attends the meeting of th [redacted] annually and serves on numerous committee for [redacted] attends at least one other meeting annually. [redacted] frequently reviews for study sections for NIH, chaired a panel for two years and served as ad hoc chair and is frequently an ad hoc member for a panel for the [redacted] also reviews papers for journals regularly, includin [redacted] serves as an associate (reviewing) editor fo [redacted] for monkey electrophysiologists.

Dr [redacted] recently [redacted] co-chaired a session on the ethics of the use of monkeys and animal welfare at th [redacted] for a consortium of international scientists and funding organizations (including NC3Rs) sponsoring a data sharing repository for best practices in neuroscience with monkeys. [redacted]).

Extensive searches are conducted for articles in our area of study. This list is updated regularly using numerous sources, including PubMed, covering the years 1960 to present. Keywords used are "population coding" and "visual perception", deep brain stimulation, Parkinson's disease, basal ganglia, target selection, vision, and saccades among many others. In addition the literature is scanned on a weekly basis, including the Journal of Neuroscience, the Journal of Neurophysiology, Science, Nature, and Nature Neuroscience and Current Biology and Neuron. Finally, most scientific journals provide an email update of table of contents (TOC) for every new issue published. Currently, the PI subscribes to email TOC for Nature, Nature Neuroscience, Journal of Neurophysiology, Journal of Neuroscience, Brain, Journal of Cognitive Neuroscience, Neuron and Current Biology.

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:

Monkey electrophysiology
Rhesus electrophysiology
Monkey in vivo Ca++ imaging
Rhesus in vivo Ca++ imaging
Monkey 2 Photon
Rhesus 2 Photon
Rhesus eye movements
Monkey eye movements
Monkey water control
Monkey alternative procedures for neurophysiology
Neuroscience alternatives in monkeys
Non-human primate and painful procedures
Periorbital eye loop implantation
Craniotomy in monkeys
Cranial window in monkeys
Head holding implants in monkeys
In-vivo microinjection
Intracranial injections
Optogenetics and monkeys
(and various combinations of these)
Single photon imaging monkeys
1 photon + monkeys
Monkeys and miniscope
2photon and monkey
Ca++ imaging monkeys
GRIN lens implants and monkeys

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.

April 1, 2020

4. Years Covered (e.g., 1980-2020):

1985- present

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.**

Animals will be evaluated for signs of pain, illness and stress by observing appetite, stool, typical behavior, physical conditions etc. by trained laboratory staff at least daily during the week. There will also be at least a daily check by the animal care staff, including the weekends. An animal will be euthanized if the animal has developed a non-treatable or incurable condition that causes significant stress or pain, as determined by the veterinarian. The animal will be euthanized according to the procedures described in appropriate section. Criteria for assessing pain or discomfort are detailed in "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research", a publication of the Institute for Laboratory Animal Research (ILAR). These criteria include increased aggression or depression, dramatic change in behavior, huddled body posture, and decreased food/water consumption. The PI as well as laboratory staff are trained to identify evidence of pain and distress in monkeys and will communicate this information immediately to the veterinarian.

In general, animals exhibiting a positive BAR (Bright, Alert, Responsive) nature are not distressed or unwell. Each animal's weight, stool consistency, urine output and food intake will be carefully monitored.
The animals will be weighed each time they are out of their cage for various reasons - for example, daily in the case of pole and collar training - but once they are fully trained, they do not need to come out daily. We will not bring the animals out of their cage every day, but rather once or twice a week at which time they will be weighed. Weight measurements are also taken prior to most procedures such as surgery and also for implant cleaning.

Weight records and water consumption records of our animals will be carefully maintained, so in the event weight changes, these data can be compared to the history of weights of the animals for veterinary decision-making purposes.

At a December 16 2019 ARC meeting, the ARC members voted to approve my proposal to be exempt from the ARC Policy on Restricted Access to Water which requires daily weight measurements for animals that have restricted access to water. Justification is to decrease possible stress caused by removing animals from cages for daily weight measurements.

Because we perform experiments that are invasive and in the brain it is possible that animals will experience brain trauma such as intracranial bleeds or infections. [redacted] and [redacted] staff are trained/assessed by DLAM to assess subtle neurological signs in monkeys that may be evident as a result of trauma. If signs of neurological impairment are present, we will inform the veterinarians of the situation immediately and discuss a course of action. In case of an emergency, to treat brain edema, Dr.[redacted] and [redacted] staff will commence immediate emergency treatment and administer one initial dose of emergency medications (as agreed upon with DLAM veterinarians). Further treatment will be continued after consultation and discussion with the veterinarians.

Because of the long term investment made in the training and care of these animals, decisions about euthanasia are made on a case by case basis together with the veterinarians.

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.

FLUID MANAGEMENT PROCEDURE

The[redacted] lab fluid management SOP outlines the steps of these procedures. In all cases when we train monkeys, we train them as much as possible without implementing a fluid management procedure. However, when tasks are more complicated and require higher levels of motivation or more consistent and reliable behavioral performance we then implement fluid control as described in our SOP. For example, we currently have two monkey that are performing behavioral tasks cage-side using a touch screen monitor system that are not on fluid control. They work for the psychological enrichment that the cognitive tasks provide.

The time of access to water will be managed in order to motivate the animals to perform their visuomotor and cognitive tasks. We also use preferred fluid (apple juice, kool-aid), but are careful to ensure this is not their only fluid due to the sugar content of these fluids.

Since rhesus monkeys are aggressive and not domesticated, the training process can be dangerous unless the animals' behavior is well controlled. Requiring the animals to perform specific behaviors to obtain a fluid reinforcer is an excellent way to ensure reduced stress for the animal and reduced risk for the experimenter. When animals have access to water as determined by their behavior, they are much more calm and are eager to learn new tasks. If new procedures are developed that can ensure the safety of the animals and the human trainers, we will happily implement them accordingly.

For untrained animals, we initially establish optimal water levels. To do this, each animal will be offered at least 100-200 ml/kg of water per day for a week in a water bottle attached to their cage to determine their optimal volume. For example, 1-2 liters of water will be provided in water bottles on the cage (nearly 4x the total volume of a typical monkey). This will be left overnight and the next day a measurement will be taken to determine how much water was drunk in that 24 hour period. Once the optimal volume (average daily intake ad lib) is established, we will begin working with monkeys and train them to drink for this volume each day. If monkeys fail to exhibit the learned behavior, or fail to move forward in the training process, we will gradually reduce the volume by 10-20-50 or 100ml steps per day until they begin to consistently perform the required behavior (either steps in the chair training process or steps in the visuomotor/decision-making tasks 1000-3000 times per day). All animals receive at least 22 ml/kg/day and most receive much more. However, in the event that we find an animal is having motivational issues, we will consult with the veterinarian to determine the cause and a course of action.

Renal evaluation will be performed on serum chemistry panels (e.g., increased BUN, creatinine, serum osmolality). This renal evaluation will be performed as directed by the veterinary staff. If all measures are normal and the monkey is still not expressing the learned behavior, the water volume will be decreased further in small steps as outlined above and will not decrease below 22 mls/kg/day.

The time in which animals receive their daily water (and possibly the volume if necessary) will be managed on this protocol to motivate animals to transfer from their cage to a restraint chair calmly and more importantly to motivate animals to perform their tasks, which can be viewed as a foraging opportunity [redacted]

Animals are rewarded with 0.1-0.3 ml of water or preferred fruit juice or water when they perform a visuomotor or cognitive task (also known as a trial) correctly. Animals typically work for 1000-3000 trials or more a day. Animals typically work to satiety during experimental sessions and the exact amount of fluid obtained varies with the size of the animal. We define satiety as the point at which animals' desire for fluid does not exceed its willingness to perform a task [redacted]. In other words, the monkey stops working in the task.

Animals that work to satiety during the period of behavioral training (2-6 hours each week

day) are provided with additional fluid in the form of a wet treat such as a piece of an orange or similar, at the end of the training/working session (1 medium sized orange contains ~60cc fluid). We will provide animals with additional 50cc of fluid at the end of each experimental session, unless this interferes with their ability to work in the lab on subsequent days.

The time that animals have access to water in the context of experimental sessions ranges between 2-8 hours per 24 hour period. If an animal only received a wet treat and no additional water at the end of the session, the time the monkeys would go without access to water is a maximum of 17 hours in every 24 hour period. For some animals, we are able to provide additional fluids at the end of the experimental sessions in the form of 'free water' (in addition to their wet treat) and they still work in the lab the next day. We do this for those animals we can. For others, we cannot because some animals quickly learn that if they receive "free" fluid in their home cages they do not perform in the laboratory, but rather they wait until they are returned to their home cage to receive their 'free' fluid. If an animal does not drink its minimum amount during the experimental session, supplemental fluid is always provided in the rig or upon return to the home cage. The minimum amount each animal drinks whether free or in the context of experiments, varies but we set a limit at slightly higher than that reported in the literature at 22mls/Kg/day [redacted]

During periods when animals are not participating in experiments, they may receive fluid ad lib, or an equivalent amount (40-100 ml/kg) in their home cages from a hanging water bottle. Monkeys in our experiments typically work for between 30-50mls/Kg/day.
Our monkeys that are > 10 years old and weigh between 8-11Kgs: the total volume they drink in the lab is between 240-400mls/day and 330-550 mls per day. Normal juvenile monkeys on ad lib water drink 380-679mls/day [redacted]
For example, we have one monkey on ad lib water that weighs 15Kgs and his optimal intake is only 300mls / day. It varies considerably by monkey.

Although sometimes monkeys may be in the lab seven days a week, it is typical for experiments to be performed five days a week. During the two weekend days, animals are provided with approximately the same amount of fluid as they obtained during the experimental session on the preceding days. This is done to avoid the possible physiological complications resulting from a sudden step to increased fluid. Therefore, when both beginning fluid access management and ending fluid access management, animals will be brought to a consistent level of daily fluid in 10-20-50-100 steps per day. We have detailed SOPs that outline these procedures to ensure the safety and well-being of the monkeys.

In the event we experience trouble motivating an animal to work, we will proceed with one overnight period with a reduced access to water. This is the only time we would 'restrict' or 'deprive' an animal's access to water. Next, the animal is brought to the lab first thing in the morning and motivation is reassessed. If the animal still does not work, we will communicate with the veterinarian who will assess the health of the animal, including blood chemistry panels and we shall follow veterinary instructions for how to proceed. Our experience is this test of motivation is not dangerous for the monkeys, as a similar restriction (< 24 hour) period is used prior to anesthetic procedures. This procedure is an excellent and sensitive indicator of the motivation, health and well-being of monkeys [redacted]

Fluid management will be conducted in full accordance with the guidelines adopted by the NIH in 1990 and updated in 2013 and those adopted by the Guidelines for the Care and Use of Laboratory Animals. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/diet_control.pdf.

These guidelines read:

"Fluid. As with food intake, whenever an animal obtains any portion of its fluid requirements through fluid reinforcers in behavioral testing, the investigator must ensure that the sum of the fluid earned through reinforcement and the supplemental fluid provided outside of the experiment is sufficient to maintain the animal in a healthy state. Experience has demonstrated that the transition of an animal to a controlled water access paradigm is best accomplished through a gradual, systematic limitation of fluid intake over a several-day period.1 If possible and consistent with the experimental paradigm, concurrent with the systematic limitation of available free-choice water, animals should be provided with an opportunity to work for additional water until satiated. In some cases, the restriction often can be relaxed or reduced after the animal becomes proficient at a given task. [6,7] However as with control of food intake, some invariant degree of fluid restriction is most often required to provide consistent motivation ensuring

reliable control over the experimental behavior. Experience has demonstrated that short periods without or with markedly reduced fluid intake may be required during the initial phases of a research design requiring water control. The duration of the period will vary with the species and hydration status of the animal. Many, larger species of nonhuman primates do well with markedly reduced or no fluid intake for periods up to thirty-six (36) hours, but smaller species, especially some New World species, (such as squirrel monkeys), may be especially susceptible to the effects of fluid restriction. Experience has demonstrated no adverse consequences of short periods without fluid intake in normal, healthy animals.[4,13,15] However, consideration must be given for the species being used and the animal's size, age, health status, body condition and concurrent testing and/or treatments. If any period without or with markedly reduced fluid intake is required after initial phases, the principal investigator should provide a clear justification for the reduced fluid intake, as well as the extent and duration of fluid reduction in his or her animal study proposal. It is recommended that at the start of a new research protocol the amount of fluid consumed, body weight and hydration assessment be recorded daily for each animal.6 Once a baseline fluid intake has been established on a given task, each animal should be allowed to earn fluids to satiety or to a level approved in the Animal Study Proposal. As needed, fluid intake should be appropriately supplemented on a daily basis. In cases in which supplements are required, the minimum amount of fluids to be provided each day should be equivalent to the amount typically consumed by the animal when it is permitted to earn fluids to satiety or to the level approved in the Animal Study Proposal. It is recognized, however, that to ensure the animal's welfare and experimental integrity, daily adjustments in fluid intake are often required during the course of the research. Once an animal has learned a behavior, the daily amount of fluid provided should be increased to the maximum level that will ensure adequate and reliable performance of the task. "Vacations" from controlled fluid intake paradigms may be advisable in some protocols. A "vacation" is a period of time, ranging from a day to a few weeks in duration, when the animal is provided unrestricted or a markedly increased fluid allocation, commonly >1.5-3 times their routine daily consumption. In studies utilizing water as a reinforcer, during periods of several weeks duration or longer, when experimental sessions involving that reinforcement are suspended, gradually increasing the animal's consumption to ad libitum access is recommended. In addition, it is recommended that animals be provided with additional access to fluid for some period on days when research procedures are not scheduled, unless scientifically justifiable reasons preclude such fluid supplementation.7 When transitioning an animal from a controlled water paradigm to ad libitum fluid [5] access, careful monitoring of the animal's dietary intake is recommended to aid in the prevention of deleterious gastrointestinal complications (e.g., "bloat" in primates, a described above for food control). Following a "vacation" period, an animal may require a period without fluid intake to regain the motivation to perform their learned task. The ACUC must balance the benefits of a "vacation" period with the subsequent need for marked fluid restriction. Assessment of Adequacy of Fluid Intake Mice and rats may be acutely water deprived for as long as 24 hours to provide motivation for drinking or tasting tests. Mice and rats tolerate this without clinically overt signs of physiologic distress or behavioral abnormalities. Each animal under fluid control must be observed daily for its health status by the animal care or investigative staff. Normal physiological responses to fluid control routinely result in robust changes in the animal's clinical pathological status. For example, fluid control will often result in elevated blood parameters (e.g., Hematocrit, Serum Total Protein, etc.), while physical and behavioral assessment of the animal indicates that the animal is healthy and adapting normally to the controlled access paradigm.[1,2,22] If at any time the attending veterinarian determines that an animal is not adapting sufficiently to the controlled fluid paradigm, the veterinarian will consult with the investigator to develop a plan to maintain the health of the animal. Even though animals typically learn to work in a manner that earns their entire daily fluid requirement during the testing session, a number of precautions must be taken to avoid the detrimental effects of fluid control. The nature (e.g., water, fruit juice) and, if applicable, concentration of the fluid reinforcement should be specified in the animal study proposal. Daily records of fluid intake must be maintained and be available for review by the veterinary staff and the institutional ACUC. The daily record should indicate the fluid earned during the testing session and any supplemental fluid and/or fruit provided to the animal. Therefore, during acute water deprivation, if water is available for a minimum of fifteen minutes per day with ad lib food, body weights records are not required.15 Similarly, food and water deprivation for periods of 24 hours or less preceded by ad lib food and water for at least 48 hours do not require body weight records. However, paradigms that require repeated periods of acute water deprivation over several weeks to months should be considered to be a chronic controlled fluid access paradigm. Some animals on a chronic controlled fluid access paradigm may decrease their total caloric intake in response to changes in their access to water. Because food intake is correlated to the amount of fluid consumed, monitoring food consumption in addition to body weight can be a valuable tool. In most cases, the decreased caloric intake is minor and does not result in a body weight loss [redacted]

fifteen percent. However, in the case of obese animals or those experiencing chronic fluid deficiency, loss of body weight in excess of 15% has been observed. This weight loss does not pose a problem in the case of obese individuals, but can lead to severe complications in the case of a chronic fluid deficiency. Therefore, as a precaution against chronic fluid deficiency during a chronic controlled access paradigm, the animal's weight must be measured and recorded at no less than weekly intervals. If an animal shows a loss in body weight of more than 15% during the period of study, when compared to the pre-diet control weight of the animal, the animal must be evaluated by a veterinarian and, if required, its fluids or food increased appropriately. Exceptions are allowed only if the attending or facility veterinarian determines that an animal is adequately hydrated and that the weight loss does not endanger the animal's health or there is a scientific justification. [6] One such exception to the above rule involves obese [18] animals which are placed on fluid restriction. When evaluating a previously obese animal with a 15% weight loss, the veterinarian may determine a weight of the animal that is closer to its "ideal" weight for the animal. In such situations, the veterinarian must clearly indicate in the animal's permanent medical record the weight to be used rather than their pre-diet control weight for future 15% weight loss assessments.

REFERENCES DENOTED [] AND PROVIDED IN
https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/diet_control.pdf

In addition to these general principles outlined by the NIH, when an animal is on managed water access, each animal will be weighed at least weekly. Typically, if an animal is taken from its cage, which could be every day, the animal is weighed at this time. Animals not involved in an experiment but on managed water access (e.g., beginning training) will be weighed weekly once trained to accept the pole. Weight is a reliable indicator of hydration since reduction of food intake is a typical sign of reduced hydration. If there are indicators of dehydration, such as dry stool or weight loss as described above, additional, quantitative measures such as hematocrit, serum chemistry, and urine/serum osmolality levels will be immediately requested of the veterinarian. We maintain a running average of daily/weekly/monthly weight records of all of our animals so in the event of a weight loss, these data can be compared to the history of weights of the animals and provided to the veterinarians for decision-making purposes.

Fluid intake and fluid provision, weight, food intake and routine animal cleaning will be monitored very closely. A daily log will (7 days a week) will be used to document the following:

•Date
•H20 provided during experiment (beginning and end times)
•H20 provided in rig or home cage
•Second access to H2O
•Weight before the experiment
•Weight after the experiment
•Dry and wet treats provided
•Cleaning: Chambers and/or skin margins- implant interface were cleaned (only in animals with head holder implants)
•Initials of personnel performing the various tasks
•Number of biscuits consumed and remaining with each of two daily feedings (generally filled in my DLAM husbandry staff).
•Time spent in play cage

We also measure and keep daily logs of the following daily observations (weekdays):

• Coat integrity (Scale 1-5: 1 being excellent)
• Urine output (1 is highly concentrated 5 extremely dilute)
• Stool consistency (1 diarrhea 5 pellets)
• Overall behavioral demeanor (assessing stereotypy). Number and 1 type of stereotypies (e.g. circling, self biting, pacing, plucking)

Food and fluid will be restricted prior to anesthesia. The restriction is necessary to prevent animals from vomiting under anesthesia. There are no adverse consequences expected from this restriction. Normal time for fasting is 8-12 hours for solid and 2 hours for fluids. To further prevent vomiting, an antiemetic may be used.

Additionally, there is a document published by the National Academy and adopted by the NIH called the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. The text of this document outlines requirements for food/fluid regulation in [redacted]

of which this protocol follows. I've copied the text verbatim below. The text in CAPS is additional text that I added to address the questions posed.

"Neuroscience-related protocols occasionally require the regulation of animals' food or fluid intake to achieve a specific experimental goal. The regulation process may entail scheduling of access to food or fluid sources so an animal consumes as much as desired at regular intervals, or restriction, in which the total volume of food or fluid consumed is strictly monitored and controlled. As stated in the Guide, "the least restriction that will achieve the scientific objective should be used" (p. 12). Research protocols that use food or fluid regulation can be divided into at least three main categories: studies of homeostatic regulation of energy metabolism or fluid balance, studies of the motivated behaviors and physiologic mediators of hunger or thirst, and studies that regulate food or fluid consumption to motivate animals to perform novel or learned tasks (Toth and Gardiner, 2000). In studies of homeostatic regulation, the manipulation of food or fluid availability would be predicted to directly influence a dependent variable that is being measured as a specific aim of the experiment, for example, food restriction leads to neurally mediated hormone release. In contrast, regulation of food or fluid is commonly used as motivation in experiments that require animals to perform a behavioral task with a high degree of repeatability (Toth and Gardiner, 2000), but the food or fluid consumption is not the experimental variable. In those studies, food and fluid regulation is used to motivate the animals to perform a specific behavioral task for a food or fluid reward; regulation of food or fluid outside the experimental session ensures response reliability to the food and fluid reward in each session
(NIH, 2002). That allows the investigator to elicit and monitor the same movement repeatedly, to present the sensory stimuli under highly controlled conditions, and to obtain physiologic discriminations from the animal. For example, water-regulated monkeys may be trained to press a button for a juice reward, while the investigator measures the effect on neuronal firing rates. In conditioned- response experiments, (for example, a monkey may be conditioned to associate a light with a fluid reward), consideration should be given to whether the use of highly preferred food or fluid as positive reinforcement can be used instead of restriction.

Fluid reward is preferable to food reward in some types of experiments. For example, studies that monitor neuronal activity in the brain may require the minimization of jaw or head movement to avoid displacing a microelectrode from its position. Because fluid rewards can be delivered through a tube positioned near the animal's mouth and tongue, they offer a particular advantage: licking and swallowing a fluid reward are much less disruptive to the neuronal recordings than chewing or crunching movements of the teeth or jaws that accompany the consumption of food rewards (NIH, 2002). Fluids offer additional experimental advantages. They can be easily delivered in small quantities, maximizing the number of trials that can be executed before satiation of the animal. In contrast with food rewards that require chewing before swallowing, fluids are quickly consumed, reducing the intertrial interval— an important advantage when an animal must perform a behavior hundreds or even thousands of times in an experimental session to allow for statistical analysis. In other studies, there may be disadvantages to using fluid rewards. For example, milk and juice require more extensive cleaning than water or solid food if spilled on the experimental apparatus. Milk and juice are also more susceptible to rapid spoilage and require frequent assessment or replacement. In designing and evaluating an animal-use protocol that proposes to regulate access to food or fluid to facilitate operant training, the following questions should be considered:

• What type food or fluid regulation is most appropriate for meeting the specific goals of the experiment?

FLUID AS WE NEED TO COLLECT MANY THOUSANDS OF TRIALS PER SESSION FOR STATISTICAL RELIABILITY

• Do alternative procedures exist that would facilitate the generation of the desired behavior without food or fluid regulation, or is food or fluid regulation the best option?

FLUID MANAGEMENT IS THE BEST OPTION TO OBTAIN THE DATA THAT WE NEED AND THE AMOUNT OF DATA THAT IS NEEDED FOR STATISTICAL RELIABILITY.

• What is the proposed schedule of food and fluid access, and does it allow periodic ad lib access to food and fluid?

PLEASE SEE ABOVE FOR SCHEDULE DETAILS. YES, ANIMALS ARE PROVIDED AD LIB ACCESS (or equivalent ~80mls/kg/day) TO FLUID PERIODICALLY AS WELL AS TREATS CONTAINING HIGH WATER CONTENT.

• What is the proposed schedule for monitoring, so adverse effects will be recognized quickly.

PLEASE SEE ABOVE FOR DETAILS.

• Is laboratory chow or fluid the only item to be offered, or will other foods or fluids be considered?
WE HAVE A DETAILED ENRICHMENT PLAN IN PLACE THAT INCLUDES WATER RICH TREATS DAILY. MANY TREATS AND CHOW ARE PROVIDED DURING EXPERIMENTAL SESSIONS AS WELL.

• What are the endpoints for intervention with supplemental feeding or hydration?"
>10% WEIGHT LOSS, EXTREME CHANGES IN BEHAVIOR AND/OR VETERINARY DIRECTION

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.

   We have a non-human primate play-cage (exercise room) that will be used regularly for the animals. DLAM staff together with laboratory staff maintain scheduling and documentation of these occurrences.

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

   Our only constraint is no wet-treats provided by DLAM. Since we control the animals' access to fluid it is important that the animals not receive additional fluid beyond what we are able to measure in the lab so the lab provides these treats to ensure they are monitored closely. It is also imperative that the people working directly with the animals be responsible for providing additional treats to establish and maintain their working relationship. As such, we provide the wet treats during the experimental sessions or immediately following the session. This is part of the training process.

   We will attempt to pair house our animals as often as possible. Often this is difficult because our monkeys are large, older males with well-established hierarchies. We have a step-wise procedure for assessing cage-mates and will implement this whenever possible. If males are paired with females, birth control will be needed. For short-term use of about 6 months or less, Depo-Provera may be used (30-150 mg IM every 1-2 months). However, for long-term pairing, a more reliable method would be to perform vasectomy on males. This will be performed by a veterinarian.

   We will always work together with the animal care and veterinary staff to pair our animals. This is critical because changing things in the animal room can lead to changes in social dynamics which could interfere with our ongoing behavioral experiments.

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 periods longer than 12 hours, or where non-USDA-covered species will be housed for periods longer than 24 hours).
3. **Research Area** (where non-surgical 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	[redacted] **Reason:** We will conduct all of our imaging procedures at th [redacted] The scanner we will utilize is th [redacted] e. Three-dimensional image acquisition and reconstruction are also available with this device.
[redacted]		Rhesus Monkey	[redacted] **Reason:** The four rooms [redacted] will be used for behavioral and Ca++ imaging experiments for testing the Siminiscope as described in the protocol.
[redacted]		Rhesus Monkey	[redacted]
[redacted]		Rhesus Monkey	[redacted]
[redacted]		Rhesus Monkey	[redacted] l **Reason:** Surgical suite where surgeries will be performed.
[redacted]	[redacted]	Rhesus Monkey	[redacted] l **Reason:** transcardial 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 selection of the most appropriate medication/agent should reflect that which best meets clinical and humane requirements without compromising the scientific aspects of the research protocol. In accordance with federal regulations, consultation 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 pharmaceutical-grade compounds whenever they are available, even in acute procedures.

If pharmaceutical-grade preparations are not available, please identify which compounds are affected and provide supporting justification in your Experimental Design. All non-pharmaceutical-grade drugs must be filter-sterilized prior to use.

Please do not list euthanasia drugs in this section.

Drug/Compound Name:	Acetaminophen
Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	10-20mg/kg
Volume:	
Frequency:	q12 hours (Do not exceed 40mgs per day).
Route:	im
Length of treatment/administration:	As needed
Purpose:	Post-Operative

Drug/Compound Name:	Buprenorphine
Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	0.01-0.03mg/kg
Volume:	
Frequency:	q6-12 hours
Route:	other: im, iv
Length of treatment/administration:	Minimum 3 days post-op, then as needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Carprofen
Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	2-4mg/kg
Volume:	
Frequency:	q12-24hrs
Route:	oral
Length of treatment/administration:	As needed
Purpose:	Post-Operative

Drug/Compound Name:	Ibuprofen
Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	5-25 mg/kg
Volume:	
Frequency:	q8-12hrs
Route:	other: oral, im
Length of treatment/administration:	Minimum two days,, then as needed
Purpose:	Post-Operative

Drug/Compound Name:	Lidocaine
Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	1-8mg/kg
Volume:	
Frequency:	As needed
Route:	other: topical, sc, im
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures Other: Minor Procedures

Drug/Compound Name:	Long Acting Bupivicaine (NOCITA)
Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	3-6mg/kg
Volume:	0.4ml/kg
Frequency:	Once
Route:	sc
Length of treatment/administration:	At incision site
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Marcaine (Bupivacaine Hydrochloride)

Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	0.1-0.5mg/kg (0.5%)
Volume:	
Frequency:	As needed
Route:	other: topical, sc
Length of treatment/administration:	As needed
Purpose:	Other: Margin cleaning, Surgical site

Drug/Compound Name:	Meloxicam
Species:	Rhesus Monkey
Medication Type:	Analgesic
Dose or Concentration:	0.1-0.3 mg/kg
Volume:	
Frequency:	q24 hours
Route:	other: oral, im
Length of treatment/administration:	3-5 days
Purpose:	Post-Operative

Drug/Compound Name:	Acepromazine
Species:	Rhesus Monkey
Medication Type:	Anesthetic
Dose or Concentration:	0.1-1 mg/kg
Volume:	
Frequency:	As needed for sedation
Route:	other: im or iv
Length of treatment/administration:	As needed for sedation
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures Other: minor procedures

Drug/Compound Name:	Clonazepam
Species:	Rhesus Monkey
Medication Type:	Anesthetic
Dose or Concentration:	0.1-1mg/kg
Volume:	
Frequency:	g12-24hrs
Route:	oral
Length of treatment/administration:	As needed
Purpose:	Other: Anxiolytic behavioral training

Drug/Compound Name:	Dexmedetomidine (Dexdomitor)
Species:	Rhesus Monkey
Medication Type:	Anesthetic
Dose or Concentration:	0.003 - 0.02 mg/kg
Volume:	
Frequency:	As needed for sedation or sedation
Route:	other: im, iiv
Length of treatment/administration:	As needed for sedation
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures Other: Minor Procedures

Drug/Compound Name:	Isoflurane
Species:	Rhesus Monkey

Medication Type:	Anesthetic
Dose or Concentration:	1-5% to effect
Volume:	
Frequency:	Continuous
Route:	inh
Length of treatment/administration:	1-8 hours
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures Other: Minor Procedures

Drug/Compound Name:	Ketamine
Species:	Rhesus Monkey
Medication Type:	Anesthetic
Dose or Concentration:	1-15 mg/kg
Volume:	
Frequency:	As needed for sedation
Route:	im
Length of treatment/administration:	As needed for sedation
Purpose:	Pre-Operative/Intra-Operative Other: Minor Procedures

Drug/Compound Name:	Midazolam
Species:	Rhesus Monkey
Medication Type:	Anesthetic
Dose or Concentration:	0.1-0.2mg/Kg
Volume:	
Frequency:	As needed
Route:	im
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures Other: minor procedures, sedation, anxiolytic

Drug/Compound Name:	Proparacaine
Species:	Rhesus Monkey
Medication Type:	Anesthetic
Dose or Concentration:	0.5%
Volume:	1-2 drops
Frequency:	as needed
Route:	topical
Length of treatment/administration:	as needed
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures Other: minor ophthalmic

Drug/Compound Name:	Propofol
Species:	Rhesus Monkey
Medication Type:	Anesthetic
Dose or Concentration:	1-12.5 mg/kg
Volume:	220 mg/kg/min
Frequency:	As needed
Route:	iv
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Other: MRI scans

Drug/Compound Name:	Cefadroxil (Cefa-Drops)
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	25mg/kg
Volume:	
Frequency:	BID
Route:	oral
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Other: minor procedures

Drug/Compound Name:	Cefaloxin
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	25-50mg/kg
Volume:	
Frequency:	BID
Route:	oral
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Cefazolin (Kefzol)
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	25-30 mg/kg
Volume:	
Frequency:	BID
Route:	other: im, iv, sc
Length of treatment/administration:	3-10days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Other: Minor Procedures

Drug/Compound Name:	Ceftiofur CFA (Excede)
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	20mg/kg
Volume:	
Frequency:	SID
Route:	other: im, sc, iv
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Cephalexin
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	10-30mg/kg
Volume:	
Frequency:	q12 hours
Route:	im
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Cirpoflaxin
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	15 mg/kg
Volume:	
Frequency:	BID
Route:	other: oral, im
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Clavulanic Acid-Amoxicillin (Clavamox)
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	12-22 mg/kg
Volume:	
Frequency:	BID
Route:	oral
Length of treatment/administration:	3-10 days
Purpose:	Post-Operative

Drug/Compound Name:	Clindamycin
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	11mg/kg
Volume:	
Frequency:	BID
Route:	other: oral im or iv
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures Other: minor procedures

Drug/Compound Name:	Enrofloxacin (Baytril®)
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	5-20 mg/kg
Volume:	
Frequency:	SID/BID
Route:	oral
Length of treatment/administration:	Extended slow release
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Erythromycin
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	10 mg/kg
Volume:	
Frequency:	BID
Route:	other: oral, im
Length of treatment/administration:	3-10 days
Purpose:	Post-Operative

Drug/Compound Name:	Metronidazole
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	25mg/kg
Volume:	
Frequency:	BID
Route:	oral
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Orbifloxacin
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	10mg/kg
Volume:	
Frequency:	SID
Route:	oral
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Other:

Drug/Compound Name:	Penicillin G Benzathine
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	30,000 units/kg
Volume:	
Frequency:	EOD
Route:	im
Length of treatment/administration:	3-10 days
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures

Drug/Compound Name:	Rocephin (ceftriaxone sodium)
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	5-25mg/kg
Volume:	
Frequency:	SID/BID
Route:	other: im or iv
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures Other: minor procedures

Drug/Compound Name:	Sulfamethoxazole-Trimethoprim
Species:	Rhesus Monkey
Medication Type:	Antibiotic
Dose or Concentration:	30 mg/kg
Volume:	
Frequency:	BID
Route:	

Length of treatment/administration:	3-10 days
Purpose:	

Drug/Compound Name:	AAV1/2-8 GCamp6F/^s 7f/7s
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	varying titers
Volume:	up tp 12 ul
Frequency:	1-2 times as needed
Route:	other: intracranially
Length of treatment/administration:	0.1ul/min
Purpose:	Other: experimental injection Ca++ indicator

Drug/Compound Name:	Atipamezole (Antisedan)
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.15-15 mg/kg
Volume:	
Frequency:	Once
Route:	other: im or ip
Length of treatment/administration:	1-2x volume of dexmedetomidine (reversal)
Purpose:	Post-Operative Non-Surgical Procedures Other: Minor Procedures

Drug/Compound Name:	Atropine sulfate
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.02-0.05 mg/kg
Volume:	
Frequency:	Once
Route:	other: im, sc, iv
Length of treatment/administration:	At induction
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Cerenia
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	1 mg/kg
Volume:	
Frequency:	30mins-2hrs prior to sedation
Route:	other: im, sc, iv
Length of treatment/administration:	Once
Purpose:	Pre-Operative/Intra-Operative Other: Minor Procedures

Drug/Compound Name:	Desferal
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.5 mg/kg
Volume:	
Frequency:	SID
Route:	im
Length of treatment/administration:	4-6 days post contrast agent as needed
Purpose:	Non-Surgical Procedures

Drug/Compound Name:	Dexamethasone
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.25-0.5 mg/kg
Volume:	
Frequency:	q6-8 hours as needed, then tapering over 7 days
Route:	other: im or iv
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures Other: Treat brain edema

Drug/Compound Name:	Dopamine HCL
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	
Volume:	2-5mcg/kg/hr
Frequency:	CRI
Route:	iv
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Other: Emergency: Increase/stabilize BP

Drug/Compound Name:	Dopram
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	2 mg/kg
Volume:	
Frequency:	As needed
Route:	iv
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Post-Operative Non-Surgical Procedures Other: Respiratory Stimulant

Drug/Compound Name:	Epinephrine
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.1-0.5mg/kg (0.5%)
Volume:	
Frequency:	As needed
Route:	other: iv, I'm, topical
Length of treatment/administration:	As needed
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures

Drug/Compound Name:	Feraheme/MION
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	8 mg/kg
Volume:	
Frequency:	During Imaging procedures
Route:	iv
Length of treatment/administration:	During imaging

Purpose:	Non-Surgical Procedures

Drug/Compound Name:	Flumazenil
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.01-1 mg/kg
Volume:	
Frequency:	Once
Route:	iv
Length of treatment/administration:	Once
Purpose:	Post-Operative Other: Benzodiazepine Reversal

Drug/Compound Name:	Gabapentin
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	10-30 mg/kg
Volume:	
Frequency:	BID
Route:	oral
Length of treatment/administration:	As needed
Purpose:	Post-Operative Other: Emergency: Anti-seizure

Drug/Compound Name:	Gadolinimum
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.2 nM/kg
Volume:	
Frequency:	once per scan
Route:	other: iv or topically
Length of treatment/administration:	
Purpose:	Non-Surgical Procedures Other: Imaging contrast agent

Drug/Compound Name:	Glycopyrrolate
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.01-0.2 mg/kg
Volume:	
Frequency:	At induction
Route:	other: im, sc
Length of treatment/administration:	At induction
Purpose:	Pre-Operative/Intra-Operative Other: Anticholinergic: Salivation Reduction

Drug/Compound Name:	Hetastarch
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	5 ml/kg
Volume:	
Frequency:	Slowly over 20 minutes
Route:	iv
Length of treatment/administration:	Slowly over 20 minutes
Purpose:	Pre-Operative/Intra-Operative Other: Plasma volume enhancer

Drug/Compound Name:	Iohexol
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	1-2 ml/kg
Volume:	
Frequency:	Once per scan
Route:	iv
Length of treatment/administration:	Once per scan
Purpose:	Non-Surgical Procedures Other: Imaging contrast agent

Drug/Compound Name:	Lactated Ringers Solution
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	5-10 mg/kg/hr
Volume:	
Frequency:	as needed during anesthesia
Route:	iv
Length of treatment/administration:	Length of anesthetic Procedure
Purpose:	Pre-Operative/Intra-Operative

Drug/Compound Name:	Mannitol
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.25-5 g/kg
Volume:	
Frequency:	Over 20mins (5g/kg) / Over 5-10mins (0.25-0.5g/kg)
Route:	iv
Length of treatment/administration:	As needed
Purpose:	Other: Emergency, brain edema

Drug/Compound Name:	Ondansetron
Species:	Rhesus Monkey
Medication Type:	Other
Dose or Concentration:	0.1mg/kg
Volume:	
Frequency:	1-2 times prior to anesthetic procedure
Route:	oral
Length of treatment/administration:	1-2 times prior to anesthetic procedure
Purpose:	Pre-Operative/Intra-Operative Non-Surgical Procedures Other: Antiemetic

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?

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:thoracotomy and exsanguination after anesthesia with barbiturates

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

Yes

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.

all animals will be euthanized

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 CO2 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:	Sodium Pentobarbital (veterinary grade)
Species:	Rhesus Monkey
Dose or Concentration:	35mg as needed to effect
Route:	iv
Purpose of Drug:	Anesthesia

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, ocular tissue

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

Blood collection is done biannually or per veterinary discretion.
Tissue collection is done once, post mortem extraction after transcardial perfusion.

In some cases other investigators would like to have tissues from monkeys for their investigations and we are interested in maximizing the use of each animal where possible. If another investigator requests this, following confirmation of death tissue will be harvested prior to exsanguination by the veterinarian. We may also be interested in performing slice electrophysiolgy on brain tissue in animals for which we have extensive behavioral data. In this case, brain tissue will be extracted immediately following the exsanguination but prior to the introduction of the fixation paraformaldehyde.

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

The typical volume of blood drawn for our animals (weighing 10-15 kgs) as needed for full blood panels and routine exams is 9-10mls.

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

Blood collection is performed by DLAM veterinary staff or trained laboratory staff. Collection occurs via the cephalic vein, saphenous vein or femoral vein and chemistry panels are performed to assess animal health and hydration status.

All tissue collection activities will be post-mortem.

The cranium is opened using a saw and the implant removed and the brain extracted.

Lab staff will train monkeys to present a limb for blood draws to minimize the number of times an animal has to be sedated as appropriate.

In some cases other investigators would like to have tissues from monkeys for their investigations and we are interested in maximizing the use of each animal as possible. If another investigator requests this, tissue will be harvested prior to exsanguination by the veterinarian.

Brain tissue collection will be post-exsanguination but possibly before introduction of paraformaldehyde for in vitro experiments. In most cases however, it is post paraformaldehyde exposure.

5. Describe how anemia and infection will be prevented.

The typical volume of blood drawn for our animals (weighing between 10 and 15 Kgs) as needed for chem panels and routine exams is 9-10mls and all items used for blood collection are sterile.

Monkeys' total volume is ~500-800mls depending on size.

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 policy requires investigators to employ the following measures to ensure asepsis while conducting survival surgery: aseptic surgical techniques; aseptic surgical field; sterile instruments; clean lab coat/surgical gown; and sterile surgical 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 duration 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 techniques must be used. Refer to the **ARC Policy on Non-survival Surgical Procedures** for further information.

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

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

- ☐ Lab tests
- ☐ Conditioning
- ☑ Fasting: 8
- ☐ Other:

Please note that a physical examination 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.

- ☑ Respiration rate
- ☑ Heart rate
- ☐ EEG

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

4. Surgical preparation of all mammalian species must include:

1) Removal of hair with #40 clipper blade in a wide margin around the incision site.
2) Three alternating scrubs using a germicidal scrub and 70% alcohol.
3) Placement of lubricating ointment into the eyes.
4) Covering the animal except the surgery site with 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 electrical 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 precautions taken to prevent hyperthermia.

A) We have a bair hugger heating system and warm water blanket for our animals. B) Animals will be provided with IV drip fluid during surgical procedures to maintain hydration.

6. Surgical preparation of the surgeon must include:

1) Wash hands with 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 mice 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) sterilization.
2) Either chemical disinfection (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, indicate the duration from anesthesia induction to euthanasia. For survival surgery, indicate the duration from anesthesia induction to recovery from anesthesia.

Survival: 5-8 hours not usually more
Non-Survival: 1-2 hours

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.

OVERVIEW

To perform these experiments we must perform:

1) implant of fiducial marker array (Minor surgery)
2) implant of head holder to replace fiducial marker array (Minor surgery)
3) Craniotomy and injection of gCAmp and implant of GRIN lens (together with the mount that holds/secures the GRIN lens). This can happen up to three times. (ie., up to three possible surgeries). It is most likely that 2) and 3) will happen in the same surgery.

Regarding the method for placing fiducial markers to perform our MRI guided neurosurgery; previously we used a non-invasive method that involved making a bite bar for our monkeys and holding fiducial markers to the bite bar. However, in our experience this method is unreliable and sometimes results in movement during surgery, reducing the precision of the MRI. As such, we may have to do one minor surgery on each monkey to install a small cranial implant to hold the fiducial markers during the MRI. This cranial implant will be later replaced with the head holder.

Thus, in total any monkey in this protocol may experience up to 2 minor surgeries and up to 3 major surgeries. If any additional surgeries are required (other than repairs as described below) we would submit an amendment.

ACRYLIC / HEAD HOLDER REPAIRS

The number of acrylic repairs is very difficult to predict a priori. I have had animals go for years without need for repair and have had other animals need repairs at 6 month intervals. If repairs requiring sedation or anesthesia for a given animal exceed 4 in any 12-month period, I will communicate in a timely manner with the ARC, together with the primary veterinarian(s) responsible or the care of the monkey, to determine if additional intervention is indicated to ensure the animal’s welfare. It should be noted that not all repair procedures involve tissue intrusion (eg. repairs to acrylic only), and some do not require sedation.

EXAMPLE REPAIRS

1) With time, the implant could become loose requiring additional screws to re-secure it. This would be a minor surgery.

2) On rare occasions (fewer since the ceramic screws) an entire implant may become dislodged. This would require a minor surgery to replace the implant.

3) The head holder or other aspects of the acrylic may break and need to be repaired. This would be neither major nor minor since no tissue is involved. If it is the head holder and we cannot stabilize the animal's head safely to reattach the head holder with acrylic, we may need to sedate the animal to reattach it.

4) Addition of a GRIN lens and mount (up to 3 total in any 1 monkey).

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

There are a number of complications that can arise associated with surgical procedures (e.g., anesthetic complications, hemorrhages, abscesses, implant failure). However, we have many mechanisms in place to minimize the likelihood of surgical complications that include:

1) All surgeries are done by experienced surgeons.

2) All surgeries are scheduled in advance with DLAM veterinarians.

3) All surgeries are performed with trained personnel in attendance, to provide anesthesia and scrub nursing support. Training is signed off on by DLAM veterinarians.

4) All surgeries are performed using aseptic (sterile) techniques.

5) All surgeries are done by fully qualified and trained personnel in anesthesia and appropriate post operative analgesics are provided to eliminate pain.

6) All surgeries are performed with pre- and post- operative antibiotic treatment to minimize the chances of infection development post surgically.

7) We use the Brainsight neuronavigation system to perform MRI guided surgeries including visualization of the brain targets and vasculature (to some extent) allowing for surgical planning that optimizes success and minimizes possible bleeding events.

8) We follow well-established procedures, vetted by veterinarians and human neurosurgeons, for handling and treating brain trauma that may result from introducing devices (used for injections or physiology) into the brain. These procedures are outlined in the animal are section.

9) We are not planning to use eye loops as the precision of the magnetic induction means this technique is no longer needed. As such we do not expect any issues associated with this procedure. None of our current monkeys have eye loops.

Food and fluid are restricted the evening prior to the administration of the anesthesic agents (at least 8 hours NPO). The restriction is necessary to prevent animals from vomiting under anesthesia. There are no adverse consequences expected from this restriction. Normal time for fasting is 8-12 hours for solid and 2 hours for fluids. An antiemetic (eg., ondansetron (0.1mg/kg)) may be administered the evening prior to surgery and/or on the morning of the procedure.

At least 1-3 days before a scheduled procedure, DLAM veterinary staff may draw blood and perform a chemistry panel to evaluate the electrolyte and renal status as well as overall health to determine suitability for surgery. Typically for animals on water management, prior to major surgery, are provided with 200-300 additional mls of water at least one day before the surgery and at least 5-7 days post surgically or longer as required for successful, healthy recovery.

ANESTHETIC PROCEDURE
Monkeys will be induced using Ketamine (1-15mg/kg), Dexmedetomidine (0.003-2mg/kg) and Midazolam (0.1-0.2mg/kg). The precise dosage will be made on a case by case basis in discussion with the veterinarians. Atropine (0.02-0.05mg/kg) or glycopyrrolate (0.01-0.2mg/kg) will be administered IM to reduce excessive salivation. The animal will be maintained on oxygen and isoflurane until intubation. One or both legs will then be shaved, disinfected and catheterized for IV injections of fluid and medications as needed. An antiemetic (For example: Cerenia 1mg/kg IV) will also be administered after IV placement.
Inert ophthalmic ointment/lubricant is placed on the eyes and the lids closed to maintain lubrication of the cornea and sclera throughout the procedure. Propofol (1mg/kg) will be used to induce anesthesia if isoflurane is insufficient. Monkeys are then intubated with an appropriate size tube.
Maintenance of anesthesia will be done using isoflurane 1-3% and oxygen 1-3l/min for the duration of the surgical procedure. Vital signs (SPO2, ECG, temperature, capnography and NIBP/IBP) will be monitored closely throughout the procedure and recorded on an anesthesia record. An IV drip is provided to maintain hydration using sterile lactated ringer’s solution (5-10 ml/kg/hr) or equivalent. The animal is situated on a heating device (bairhugger) and the temperature of the animal is monitored.
Because it is often difficult to assess whether animals can breathe voluntarily during a surgery when ventilated, we have moved away from automatically ventilating animals during surgery. However, if determined by the veterinary or surgical anesthetist that ventilation is required, automated ventilation may be used.

ANALGESIA
Local anesthetics: lidocaine (1mg/kg) and bupivacaine (1mg/kg) will also be administered along the incision site at the start of surgery.
Buprenorphine (0.01-0.3mg/kg) will be administered IV at the start of the surgical procedure and then q6-12hrs for the first 24-48hrs depending on the severity of the surgical procedure and in discussion with the veterinarians on a case by case basis.
Meloxicam (0.1-0.5 mg/kg PO), ketoprofen (1-2 mg/kg IM/SQ/IV) or flunixin meglumine (1.0-2.0 mg/kg IM) will be provided for at least 48-72hrs post surgically. If further pain management is needed, a veterinarian will be consulted.

ANTIBIOTICS
Antibiotics that are known to have high dural penetration and cross the blood brain barrier are provided prophylactically at least 12 hours before surgery and continue for the duration required by the particular antibiotic, typically 3-14 days. The following

antibiotics may be used: Excede (20mg/kg SC), Cefa-Drops (25 mg/kg, PO), Cefadroxil (25 mg/kg), Cefazolin (25 mg/kg, IM or IV), Clindamycin (11mg/kg, PO, IM or IV), Rocephin (Ceftriaxone sodium 25 mg/kg, IM or IV), Metranidazole (25-30mg/kg PO).

STEREOTACTIC PLACEMENT FOR PRECISION GUIDED NEUROSURGERY

Head stabilization for neurosurgery may be performed in one of two possible ways. The first is using a traditional David Koph stereotactic device (Horsely Clark Method). The second is using a 'halo' as is done in human neurosurgery and developed by the Montreal Neurological Institute and Rogue Research Inc. for monkeys.

Horsely Clarke Method:

The animal is positioned in a stereotaxic frame facing forward and the head is immobilized with the stereotactic frame. Topical anesthetic lidocaine or marcine ointment may be applied to the ear bars to minimize any pain or discomfort. This process can take up to 15 - 30 minutes depending upon the animals' anatomy and the experience of the individual. It sometimes takes longer if the PI is training new people to perform this procedure.

OR

C-Clamp Method:

The animal is positioned in a C-clamp, which suspends and immobilizes the head using 3-4 pins secured to the skull and the clamp. Then pins penetrate the skin and push against the bone but do not penetrate bone, only skin (which is usually performed by a stab incision using an 11 blade). An identical system is employed in human neurosurgery. This procedure takes up to 15 - 30 minutes depending upon the experience of the person performing the procedure. It takes longer when the PI must train new people to perform the procedure.

HEAD HOLDER IMPLANT/ FIDUCIAL MARKER IMPLANT

To ensure the stability of the head required for eye movement recording accuracy and behavioral/cognitive assessment, we may implant a head holder to the skull of the animals. We will do this if the head stabilization with mask approach fails. For the implantation of the head holder, animals are anesthetized and prepped as described above and placed in the David Koph stereotactic device as described above. An incision is made in the scalp and the periostem retracted/removed. Next 10-20 (depending on the size of the animal) ceramic bone screws are implanted into the skull. A hand drill makes the burr hole which is then tapped and the screw is installed using specially designed surgical instruments. Then, using a stereotactic arm holder, the head holding implant (an ~ 7mm diameter delrin hollow tube) is secured to the skull and the bone screws using dental acrylic. The head holder is sealed in place and the acrylic is smoothed to the edges of the skull to ensure healthy healing and reattachment of the scalp to the implant margin. Sutures or wound clips are applied to the scalp as needed to close the wound and ensure proper healing. The same procedure is used for the fiducial marker implant prior to MRIs to ensure accuracy of the MRI during the surgery. The fiducial marker is smaller in diameter and is held by 2-4 screws and will be removed when the head holder is place (in the same location).

GRIN LENS MOUNT INSTALLATION:

After an initial craniotomy (<2mm) used to introduce AAV GCamp using a 10ul Hamilton syringe mounted to the arm of a stereotaxic frame, the mount that is required to secure the GRIN lens in place is installed. The goal of this proposal is to develop the surgical techniques and the devices required to install a a mount securely to protect the craniotomy and to allow implantation and attachment of the GRIN lens and Siminiscope to the skull. Based on our experience with 1 monkey this past year, we redesigned our mount and made it entirely of glass reinforced PEEK material so that once installed MRIs will be possible. Also we designed a new skull helmut that will be placed over the mout to protect the monkey and the mount(s). Up to three craniotomies and associated injections and mount installations will be performed in any animal.

NON-SURVIVAL CARDIAC PERFUSION/EUTHANASIA

After being sedated and anesthetized with isoflurane (as above) the monkey will be deeply anesthetized with sodium pentobarbital (at least 35 mg/kg, iv) and once all reflexes are absent the sternum will be opened using sharp dissection, scissors and rib

cutters. At this point, other tissues may be harvested for use by other investigators or for necropsy. Next, the descending aorta will be occluded by clamping with 1-2 pairs of hemostats. The pericardium will be opened to visualize the the left ventricle and the right atrium. The ventricle will be cannulated and the atrium will be cut. First, warm phosphate buffered saline containing 0.125% glutaraldehyde and at least 2 liters, will be introduced through the left ventricle and drained from the right atrium. At this point brain tissue for in vitro experiments may be harvested. Next, at least 2 liters cold 4% paraformaldehyde will be introduced for tissue fixation. Death occurs by thoracotomy and exsanguination. The process takes between 2 and 4 hours.

Note: All medications and drugs provided to monkeys will be pharmaceutical / veterinary grade, except the AAVs which are quality controlled by the UPENN, UNC or Salk vector cores.

11. Please indicate the suture materials to be used:

- ☑ Internal: absorbable sutures (e.g., Dexon, Vicryl)
- ☑ 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 attention should be given to thermo-regulation, cardiovascular and respiratory function, and post-operative pain or discomfort during recovery from anesthesia.

Endotracheal tubes will be removed when laryngeal reflexes return. Animals will be monitored closely by veterinary, animal care staff or lab staff, and observed continuously until BAR. The continuous observations are recorded every 15 minutes until fully recovered from anesthesia. Full recovery is defined as when the animal is able to sit upright unassisted.

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

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

Animals undergoing survival surgery will be allowed to recover from surgery in their home cages. Patient body temperature will be maintained with a heat lamp. Food and water will be returned when animals are sufficiently ambulatory. For all procedures animals must be monitored closely for the first 48hrs post operatively, a minimum of two checks per day must be performed. Minor procedures will be monitored for up to 10 days total which can be reduced or increased on a case by case basis through discussion with the veterinarian, and is dependent on how well the monkey is doing. For major surgical procedures (e.g. craniotomy) there will be a minimum of a 10 day post op monitoring period in which the animals are monitored at least twice daily. All post operative monitoring will be done by DLAM staff and/or laboratory staff and records of the animal, its surgical site and medications received, are recorded.

Species Surgery

Species:	Rhesus Monkey
Number of Animals:	15
Surgery Type:	Multiple Survival Surgery
Surgeries per Animal:	5
Time Between Surgeries:	2 weeks -6 months

Non-Surgical Procedures

1. Describe the basic methods used for all non-surgical manipulations (e.g., imaging, behavior)

studies, Parkinson's and diabetes induction, chronic implant maintenance, cannulation).

IMAGING PROCEDURES

Non-invasive methods for visualizing locations of specific brain regions include:
- Magnetic Resonance Imaging (MRI)) together with contrast enhancing agentWill be used to assess regulation of neurotransmitter systems such as dopamine. Monkeys will be fully anesthetized during imaging procedures.

A commercially available stereotaxic device that is non-ferromagnetic (titanium, brass, and plastic) may be used to stabilize the animals' head while inside the MRI unit. We may also use a specially designed Brainsight system in which case the stereotaxic device is not required and the animals' head is held stationary by the already implanted head holder or by placing cushions and blankets along the side of each ear of the animal or by the fidicual marker holder previously implanted.

MRIs will be used pre-operatively to guide placement of craniotomies and injections and post operatively as needed to assess efficacy of chamber placement.

MRI
The critical aspect for the pre-surgical MRIs is having fiducial markers placed that can be imaged together with the head and then replaced at the time of surgery. This aspect is critical to ensure the accuracy of the brain targeting. We have two methods to place the fiducials. The first is by making a custom bite bar for each monkey. To do this, the monkey is lightly sedated with Ketamine and Dexmedetomidine, and a melted hydroplastic mold is placed in the mouth to create a hard bite bar. The bite bar has attachments that hold fiducial markers. So during the MRI, the bite bar is placed in the monkey's mouth and the fiducials that can be imaged alongside the monkeys' head. We've performed this approach previously to mixed results - the accuracy and the ability to recapitulate the mouth bar's position in surgery as it was during the MRI is very difficult. An improved method requires the implantation of a small head holder on the skull that allows for the attachment of the fiducials whose position can be recapitulated identically at the time of surgery (described in surgery section).

For the MRI procedure, monkeys are induced and anesthetized as described for surgical procedures and are transported using a specially designed transport chamber that allows for full monitoring of the animals' vitals during transport while under isoflurane anesthesia. Using this USDA approved method of transport (LAB SOP 19 Non-Human Primate Transport), the animals are brought to the [redacted] where they are placed in the MRI to be imaged. Animals are catheterized in a leg vein to allow for the introduction of contrast agents such as gadolinium or iohexol or feraheme (LAB SOP MRI ; [redacted]
After the scanning process (1-2 hours) animals are returned to the transport box and returned to their home cages where they are recovered, monitored and their health status recorded every 15 minutes until ambulatory.

POLE AND COLLAR AND CHAIR RESTRAINT TRAINING AND USE

The two most popular types of collars available are a rigid type designed for the 2-pole technique and a flexible collar made of chain link. In the 2-pole technique, one pole is attached to each of the two sides of the rigid collar that is around the monkey's neck. I use the two-pole technique only in two instances. One, when the animal is poorly trained and very large and two people are required to handle the animal. In the second, if I am training someone on the technique and they require added assistance from someone more experienced (i.e., they are not yet comfortable handling the monkeys alone). Typically, the flexible collars consist of a single, stainless 'rope' (a chain link). Sometimes this is covered with plastic, but often it appears more comfortable as a smooth metal chain.

The collars are fitted around the neck of the monkey under light sedation (dexdmedetomodine/midazolam). The collars are monitored regularly for safety and fitting comfort and are easily adjusted for comfort and safety by either adding or subtracting chain links.

The collars are equipped with an additional larger ring where a restraint pole can be attached. The collars are flexible so that the pole can be attached 360 degrees around the collar, making the polling process faster and therefore less stressful for the animals. Although once trained, the technique is not stressful for the animals.

Once fitted with a collar, each animal is acclimated and trained to accept the attachment of a rigid restraint pole to the collar using positive reinforcement. Restraint poles are

36 inches long and rigid and have a lockable clip on the end of the pole, which clips on the collar. Once acclimated and trained to accept the pole calmly, monkeys are then trained to exit the cage calmly and walk on the end of the pole, guided by the human/trainer.

Once the animal has learned to move calmly out of its cage, it may be trained to move to a scale where it will be weighed each time it is taken out.

Next we train the animals to enter a primate chair. Our primate chairs are a mobile, plexiglas restraint device that encloses and restrains the animal and protects staff working with the animals. Monkeys can move comfortably inside the chair, but they cannot reach outside of it. The chairs are equipped with an adjustable neck plate that completely encloses, but does not restrain, the animal's arms within the chair leaving only the head exposed. With this plate, the animals cannot reach outside of the box, avoiding the potential of injuring themselves, and/or the humans working with them.

Although individual animals differ, the typical time required for the pole, collar and chair training is around 2 weeks, 4 being the maximum. We have a detailed SOP for our training procedures that are highly effective and are optimized to maintain the health and safety of the animals and their handlers.

Our procedures use only positive reinforcement based on the method of successive approximations as outlined by BF Skinner. If you view the behavior of accepting the pole, exiting the cage and entering a chair as a sequence of smaller behaviors, each step in the chain of behaviors is done in a step-wise fashion. At the end of training the individual steps are strung together to make the behavior of getting into the chair. We monitor the animals' behavioral performance with each of the steps. Once one step is learned we move to the next.
Motivation is a key aspect of the training [redacted] and we use fluid motivation as it is the most successful and safe for the animals.

All restraint training will be performed in accordance with the regulations of the Animal Welfare Act and the recommendations of the Guide for the Care and Use of Laboratory Animals. During training, animals enter an exit the chair multiple times in a single training session depending upon how quickly they learn to do this. The typical number of times animals are in the chair during initial training is for approximately one hour, no more than 3-4 times per day.

Once accustomed to entering and exiting the chair, animals are rolled into the procedure room in the chair to cognitive and behavioral testing described below. Animals are allowed to become fully habituated to the procedure room before beginning training on the performance of behavioral and cognitive tasks. Details of all our procedures for these processes are outlined in our laboratory SOPs.

EXCEPTIONAL TRAINING CASES

In some cases (once in 20 years), monkeys show behaviors consistent with a higher than typical amount of anxiety during the training process. For these cases, we will inform the veterinarian and implement what I refer to as an extinction trial. In this case, we would provide a low dose of an anti-anxiety medication to the animal prior to a training session. Next, we would commence the training session while the animal has the 'edge off' to let it know that the chair is a safe place. In my experience this kind of addition to the training is only required once or 3 times maximum and the animal reduces its anxiety behaviors.

EXCEPTIONAL CHAIR RESTRAINT CASES

In exceptional cases (1 monkey in 20 years) monkeys learn a behavior that puts them at risk in the chair. They learn to shake or move their bodies around in the chair. If they do this, because they are so strong, this behavior puts an unsustainable level of strain and torque on the head holder, putting it at risk of breakage.

We had one monkey [redacted] who did this sometimes and as a result broke his head holder 3 times. The repairs were minor (requiring only a re-acrylic) but they also required sedation. To minimize the chances of any further breaks while finishing the experiments, we placed an additional restraint inside the chair. It is a waist collar not unlike the neck plate, that minimized his ability to rock the chair. This was designed to fit comfortably around his waist with a soft cushion to avoid creating discomfort or abrasion.

Dr. [redacted] is working closely with an engineer to design new chairs for use in the lab. With these chairs, which have a lift device, we are able to wheel the chair adjacent to the door of the home cage allowing for monkeys to be jumped directly from the cage to the chair. This would eliminate the need for pole and collar training, a significant refinement and also a significant reduction in training time leading to experiments.

[redacted]

BEHAVIORAL AND COGNITIVE ASSESSMENT IN THE LABORATORY

After pole and chair training are completed, monkeys will be trained to perform visuomotor tasks ranging from fixation to complex decision making. For example, in a fixation task, a spot of light will be projected at the straight ahead position on a screen that is in front of a monkey. The monkeys' task will be to maintain gaze on this spot of light for a period of time (500ms - 5s). The monkeys' gaze must remain accurate for a predetermined time period and within a particular distance from the spot of light which is ensured by measuring the eye movements with the infrared tracking technique. When the eye position stays within a predetermined position for a predetermined period of time, a drop of fluid (water or fruit juice; 0.1-0.3 ml) is provided as reward via an automatic sipper device positioned close to the animal's mouth. After a brief delay (~1s) the next trial begins anew. Motivated animals typically work for 1000-3000 trials or more a day (i.e., 100-300 ml of water/juice). Typically for easy tasks, monkeys can receive between 2.5-5ml of fluid in a 5 minute period.

In complex decision-making tasks, monkeys start off with the fixation task. After a period of time the spot of light that appears in the center of the screen is joined by the appearance of 2-4 additional spots of light at other locations within the visual field. The central spot of light is then replaced by a patch of 'white noise' (looks like snow on your television screen but stationary). These stimuli are called Glass patters if orientation is the visual cue or random dot motion stimuli if motion direction is the cue. We can vary the difficulty by change how coherent the 'white noise' looks. The monkeys' task is to make a decision about the orientation or the motion direction and to report the outcome of that decision process by making an eye movement to one of the target spots in the visual field.

Throughout the daily task sessions, the animals will be allowed periodic "breaks" during which they may be supplemented with treats (e.g., dried fruit, nuts). Animals' head may be unrestrained during this period if in the judgment of the experimenter it is safe for the animal (animal cannot access implant devices and hurt itself). Animals indicate the time at which they would like a treat when they fail to fixate the visual target reliably indicating a "break time" or a desire to be returned to their home cage. At this point, animals will be given a treat or will be returned to their home cages, or both, being careful not to provide rewards for behavior unrelated to the experimental task. Because monkeys tell us when they no longer wish to perform the task and they are free to make postural adjustments in the chair - we view this as escapable, partial restraint.

EYE MOVEMENT AND EYELID RECORDING

While monkeys are seated in the primate chair they perform video games. We monitor their eye movements to record their decisions and choices using the non invasive infrared eye movement tracker, which consists of a camera mounted to a table placed in front of the monkey. We may also measure the movements of the eyelid using the magnetic induction technique. A small diameter (~4mm) loop of insulated wire is attached with false eyelash adhesive to the lower margin of the upper eyelid. This procedure is non-invasive and is tolerated well by monkeys, with habituation. The same procedure is performed on humans to monitor movements of the eyelid and is not painful and does not restrict the eyelid or obstruct vision. The loop can be removed by simply removing the false eyelid adhesive akin to removing a band-aid. To habituate monkeys to this procedure, treats are provided while applying and removing the lid loop. The habituation plan is simply approaching the animal slowly, letting it see the loop and tape without touching or biting it and then slowly reaching the animal's eyelid. Treats are provided the entire time. Next the tape is gently secured to the lid. This process takes about 10 minutes. The tape is removed gently as you would a bandaid. The loop and tape are very small and very light and do not interfere with normal lid closure (if it did it would preclude our measurement).

CA++ IMAGINING PROCEDURE
While monkeys are seated comfortably in their chairs and playing video games, the helmut will be removed and the Siminscope will be secured to the top of the GRIN lens/ mount system. This is all non invasive and is not painful for the monkeys. THe Sinimiscope is attached to a computer and digital images of brain activity (through the GRIN lens) is

are acquired while monkeys play video games and earn fluid rewards.

CLEANING PROCEDURE

For 1-2 weeks after surgery, the skin/explant margin will be left to heal. Typically no cleaning will occur during this time. If necessary, as determined by daily examination, the margin may be rinsed with, for example, sterile saline as needed. The recording cylinders will be flushed with sterile saline as needed during this period. For routine maintenance, the implant edge will be maintained with sterile saline or povidine solution as needed. Monkeys tolerate this well in my experience after using a formal habituation procedure.

Changes in salivary cortisol during this procedure does not exceed those seen during typical diurnal fluctuation [redacted] Animal do differ in sensitivity therefore, we may apply a topical analgesic solution (lidocaine, bupivacaine) to the margin to minimize sensation if needed. It is not advisable to place moist items such as creams on the margin since that promotes bacterial growth. Moreover, an injectable analgesic would introduce pain in an otherwise not painful experience, therefore topical treatments may be applied. These treatments include Nolvasan (chlorhexidine acetate 1.0%) or Prontosan Wound Irrigation Solution as a disinfectant, and silver sulfadiazine (SSD) cream (1.0%) pr granulex (trypsin) to assist healing. The cylinder(s) will be flushed with sterile saline and povidine/betadine solution daily during ongoing experiments and on an as needed basis. When animals are engaged in experiments, they will be cleaned as many days as experiments are performed depending on the condition of the explant and the surrounding skin. When animals are not engaged in experiments, they will be cleaned at least 3 times per week (usually Monday, Wednesday and Friday as needed). We have a rigorous cleaning procedure that is implanted as conditions require. See SOP.

Each cleaning will be documented in a cleaning log maintained in the laboratory. It is expected that the chambers will become colonized with bacteria commonly found on the skin of the animal. Swabs of the discharge from the cylinder and the skin/explant will be cultured when deemed necessary and appropriate antibiotics, anti-inflammatories, and analgesics will be administered as necessary.

In some cases, to extend the expected life of the chamber implants, we will seal the chamber with a silicone 'plug' (either Kwik-Sil from WPI. or Vaseline) to provide a sterile seal and minimize the frequency of cleaning. They will be checked at least weekly to ensure the seal remains closed. This procedure can be done anytime during the lifetime of the anima [redacted]

As a second refinement we will also try is to maintain head stability in monkeys using a hydroplastic mask. For this, a facial mask will be molded to the animals head under light sedation. This mask will then be affixed to the chair and the monkey will be trained to insert its head into the mask for eye movement measurements. In principle this procedure can be used for electrophysiological recording as well, but it is far riskier as animals could be hurt with the recording apparatus attached and if they move their heads. We will trial this method [redacted]

In an effort towards refinement, we will also begin training all of our monkeys to present a limb for blood draws as prescribed by the veterinarian. This process is like all other training processes in which positive reinforcement is used to provide read for small behaviors leading to the eventual behavior of limb presentation. For the training, once we have established comfortable limb presentation, we will not insert a needle each time, but rather perform the process of shaving and compression to allow for clear visualization of the vein of interest. We will perform only 2 - 3 actual sterile needle insertions (with saline) to ensure the animal is trained.

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

As far as we are aware there are no potential complications of non-invasive imaging techniques per se, the risk is in the anesthesia. All training methods use positive reinforcement and the method of successive approximations so the possibility of experiencing stress during training pole and collar and chair training procedures is minimized.

Gas Anesthetic

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

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

- ☐ Halothane
- ☑ Isoflurane
- ☐ Other: prop

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

- ☐ Certified Fume Hood:
- ☑ Other: charcoal filter

Scavenging Location

Building	Room
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
[redacted]	

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
adeno-associated virus	intracranially	0.5 - 5ul	n.a.	4/17/2020
atropine	IM SC IV	max 1.25cc	n.a.	3/23/2018
midazolam	IM	max 0.64cc	n.a.	3/23/2018
paraformaldehyde	transcardially	2-4 liters to effect	post exsangination	3/23/2018

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:

In order to measure eye movements and record brain activity, we must have the animal remain stationary with its head stabilized. The enclosure of the chair not only facilitates the experiment but protects the animal from reaching out and touching devices that are placed during the experiment, such as the Siminscope used for Ca++ imagining.

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

Custom designed primate chairs are used. They are sized for the animals to be able to sit comfortably on their haunches. They have room to move their bodies, make postural adjustments, even when the head is held in position (partial restraint). Animals are trained through a method of successive approximation procedures to be taught how to accept the pole for restraint and then to learn how to get into the chair that is used for restraint. Chair conditioning could take anywhere between 2 days to 2 weeks, depending on the animal's motivation level. Once trained, in my experience, animals willingly get on to the pole and treat the chair as if it were another home cage. The restraint of the chair

is also escapable since if monkeys no longer wish to participate in experiments, they do not engage in the task (for the video games requiring eye movements).

3. Restraint Duration and Frequency:

NOTE: The period of restraint should be the minimum required to accomplish the research objectives.

Animals are transferred back and forth between the chair and cage using a removable, rigid pole attached to a standard chain-link collar or a version consisting of a single, thin rubber casing over a stainless "rope" attached to the animal. Animals rapidly adapt to wearing these collars. For comfort, these collars contain a rubber coating to minimize abrasion between the collar and the neck skin and provide rigidity to the collar. Occasionally, the rubber coating is irritating to the underlying skin and has to be removed. The collars that we recently purchased also eliminate this as rubber coating is higher quality material and more durable. Each time an animal is removed from its cage, the skin under the collar will be evaluated and the collar will be adjusted appropriately or removed if abrasions are present. Appropriate care will be administered (e.g., disinfectant, topical or systemic antibiotic) if a wound is present. We monitor the collars so closely that we do not expect abrasions to occur. Also, for the chain link collars there are additional links on the collar that allow it to be lengthened to compensate for the increases in neck size that may occur with the aging of the animal.

With the implementation of the new chairing procedure, the pole and collar method of handling and restraint will be eliminated completed.

Animals will remain in the chair for the experimental period (not to exceed 12 hours) or for implant inspection and maintenance or observation by the research/veterinary staff. During experimental sessions, typically lasting a total of 3-6 hours, an animal's head will be stabilized but the body is free to move within the chair for normal and periodic postural adjustments. During transport from the home cage room to the experimental suite, the head is mobile. During the experimental session, animals are monitored using an infrared camera. A person will remain with the animals at all times during experimental sessions or when in the chair.

Animals dictate the duration they wish to be in the chair. An indication that animals no longer wish to participate is that they no longer engage in the behavioral task (escapable restraint). The visuomotor tasks described in this protocol require animals to maintain their gaze on visual stimuli. An indication of the desire to no longer participate is given when the animal no longer looks at the stimulus.

4. Describe how frequently the animals will be observed during the restraint period.

Please also describe criteria for removal of animals from restraint.

Virtually constantly. We have an infrared video monitor that observes the animals throughout the experimental session. Recording of eye movement signals as well as signals from the brain are also performed continuously.

Daily chair training sessions can last anywhere from 1 - 3 hours depending on the animal. They may also occur multiple times in one day to reinforce learned behaviors (or to work on extinguishing bad ones). Monkeys are not in the chair the entire time during training. They enter and exit repeatedly to train and reinforce calm entry and exit from the chair. We always end the training process on a positive note (the animal successful performs the desired behavior and received a reinforcer).

If restrained animals appear to be under stress for any reason (e.g., rough coat, vocalizing, persistent struggling), they will be removed from restraint for a minimum of 2 hours before another attempt at restraint is made. If animals continue to show signs of stress during restraint (e.g., after up to 5 days of attempts of training a behavior) a procedure will be attempted that allows for administration of an anti anxiety medication to relax the animal and resume training. I view this as a final effort before deciding that the animal cannot be trained. In the 20 plus years that the PI has been working with this animal model, the PI has never had the experience of not being able to train an animal and has required this approach of using an anti-anxiety medication only once.

5. Will pain or discomfort be induced?

No

Species Restraint

Species	Number of Animals
Rhesus Monkey	15

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:

Yes	No	
◉	○	I agree to abide by all applicable federal, state, and local laws and regulations and UCLA policies and procedures.
◉	○	I am aware that deviations from an approved protocol or violations of applicable policies, guidelines, 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 supervision 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 application is accurate to the best of my knowledge. If this project is funded by an extramural source, I certify that this application accurately reflects all currently planned procedures involving animals described in the proposal to the funding agency.
◉	○	Any modifications to the protocol will be submitted 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 submitted for review and approval of the ARC prior to initiation.

Completed by [redacted]

FS Assurance

This section is empty.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN 53715

[redacted]

INVOICE

Date: December 12, 2018 **Invoice Number:** [redacted]

To: UCLA - [redacted]
ATTN: [redacted]
[redacted]
EMAIL: [redacted]

Re: PO# 2000PWF176
Amount Due: $19,452.00

Qty/Unit	Description	Unit Price	Extended Price
4	Macaca mulatta (Rhesus), of Indian Origin 2 Older Males @ $4,500 each 2 Young Males @ $5,000 each Lab Testing: $400 Fed Ex Charges: $ 52	$ 19,452.00	$ 19,452.00
		TOTAL DUE	$ 19,452.00

Shipped: November 28, 2018

PLEASE MAKE PAYMENT TO:

[redacted]

Invoice questions may be directed to:

[redacted]

UCLA
Division of Laboratory Animal Medicine
DLAM | Veterinary Services
[redacted]
ccredited by AAALAC, Int'l. since 1976

Physical Exam Form

Protocol# [redacted]	Animal #	BH41

Physical Examination

[redacted]

Date:	12/14/18	Examined By:			
Body Weight:	11.6 kg	Heart Rate:	120	Respiratory Rate:	20
Temperature:	99.8 F	CRT:	<2	MM:	pink
BCS:	5/9				

CRT-Capillary Refilling Time, MM-Mucous Membranes, BCS-Body Condition Score

O

Body System	N	A	NE	Body System	N	A	NE	Body System	N	A	NE
1. Integument		✓		5. Eyes	✓			9. Ears	✓		
2. Musculoskeletal	✓			6. Cardiovascular	✓			10. Respiratory	✓		
3. Gastrointestinal	✓			7. Urinary	✓			11. Reproductive	✓		
4. Endocrine			✓	8. Nervous	✓			12. Lymphatic	–		

Test	N	A	ND	Specify Test	Test	N	A	ND	Specify Test
Complete Blood Count					Parasitology				
Chemistry Panel					Culture				
Urinary					Other:				

N- Normal, A- Abnormal, NE- Not Examined, ND- Not Done

Veterinarian Follow-up

A

Clinical Notes/Test Results:

1-1 Diffuse alopecia
& Natural substitution and excessive
Needs dental

P

Follow-up/ Treatment:

1. QD
HBV serology
BC
Chem
Ivermectin
Appointment
Placed collar by PI (client)

① corrected protocol

Final Review By (DVM) [redacted]

California Department of Public Health

CDPH

Permit No. 337

Date Issued 11/27/2018

Research Importation Permit

ISSUED TO: University of California, Los Angeles [redacted]

The application dated 10/23/2018 for a permit to import specified wild animals is hereby granted, subject to the provisions of the California Code of Regulations, Title 17, Section 30070-30086 and to the terms and conditions stated below.

1. **Specified wild animals being imported:**

Quantity	Common Name	Scientific Name
4	Rhesus macaque	*Macaca mulatta*

2. **Terms and conditions under which the above species are permitted entry:**

Four (4) animals (ID Nos. AT03, AV63, BH41, BI25) to be imported.

3. **Place and conditions of quarantine:**

University of California [redacted]

4. **Name & Address of Attending Veterinarian**

[redacted]
University of California
[redacted]

5. **Name & Address of Consignor**

University of Wisconsin
[redacted]

6. **Quarantine provisions:**

See attached *Guidelines for Nonhuman Primates Imported for Exhibition and Research*

AUTHORIZATION
VETERINARY PUBLIC HEALTH SECTION

Name: Curtis L. Fritz, DVM, PhD
Title: State Public Health Veterinarian
Date: 11/27/2018
Total: $40.00

Copies to: Importer or Agent ☑ Co. Environmental Health/County or PH Veterinarian ☑ Attending Veterinarian ☑

Veterinary Public Health Section/ Division of Communicable Disease Control
MS 7308, P.O. Box 997377, Sacramento, CA 95899-7377
(916) 552-9740
Internet Address: www.cdph.ca.gov

California Department of Public Health CDPH

CDPH Use Only

Permit No.

Record No.

Date Issued:

IMPORTATION OF SPECIFIED WILD ANIMALS INTO CALIFORNIA VETERINARY CERTIFICATE OF QUARANTINE

INSTRUCTIONS: An attending veterinarian authorized to practice veterinary medicine under provisions of the California Business & Professions Code, Chapter 11, Division 2, must submit this completed form to the Veterinary Public Health Section (VPHS), and have received written authorization from VPHS prior to releasing animals from quarantine. Please complete the form and print, sign, scan, and email to VetPH@cdph.ca.gov or fax to the number listed below.

	Name	Address	Email Address	Phone
Veterinarian	[redacted]			
Owner/Agent				

Common Name	Scientific Name	Entered Quarantine Date	Quantity	Proposed Release* Date	Quantity
Rhesus Macaque	Macaca Mulatta	11/29/18	4	1/15/18	4

* Attending veterinarian must possess written authorization from VPHS prior to the physical release of animals.

Summary of other findings, including additional tests, physical examinations, illnesses/deaths, etc.

All animals arrived in good health. Results of physical exams, CBC/chem and fecals were within normal limits. Results of B-virus tests were negative for all. There were no signs of illness during quarantine.

[redacted]

Veterinary Public Health Section, MS 7308, PO Box 997377, Sacramento, 95899-7377
Phone: 916.552.9740 Email: VetPH@cdph.ca.gov Fax: 916.552.9725

VETERINARY CERTIFICATE OF QUARANTINE

Tuberculin Testing Summary Report
Non-human Primates

Common Name:	Rhesus Macaque			
	INITIAL TEST		**RETEST**	
Test Date	12/4/18		1/10/19	
Type of Antigen Used	Tuberculin Mammalian Human Isolate		Tuberculin Mammalian Human Isolate	
Final Concentration in mg/0.1mL	0.1ml		0.1ml	
Manufacturer	Colorado Serum Company		Colorado Serum Company	
Lot Number	445x		445x	
Number of Animals Tested	4		4	
Injection Site	OD		OS	
Tuberculin Reaction	**# Positive**	**# Negative**	**# Positive**	**# Negative**
24-hour	0	4	0	4
48-hour	0	4	0	4
72-hour	0	4	0	4

CERTIFICATION

I hereby certify that the animals imported under Permit 337 issued by the California Department of Public Health have completed the quarantine set forth under applicable provisions of the California Code of Regulations, Title 17, Sections 30070-30086, under my supervision. I have examined said animals and find that those to be released from quarantine are free from signs of contagious or infectious disease

[redacted]

DVM reviewed
JAN 15 2019

[redacted]

Please email this signed Certificate of Quarantine to VetPH@cdph.ca.gov

Veterinary Public Health Section, MS 7308, PO Box 997377, Sacramento, 95899-7377
Phone: 916.552.9740 Email: VetPH@cdph.ca.gov Fax: 916.552.9725

California Department of Public Health

CDPH

Permit No.: 337

Date Issued: 11/27/2018

CERTIFICATE OF RELEASE FROM QUARANTINE OF SPECIFIED WILD ANIMALS

In accordance with the provisions of the California Code of Regulations, Title 17, Sections 30070 through 30086, a certificate of release from quarantine of specified wild animals is hereby granted.

Attending Veterinarian	Owner/Agent
[redacted] University of California [redacted]	University of California, Los Angeles [redacted]

Animals to be Released:

Common Name	Scientific Name	Date	Quantity
Rhesus macaque	*Macaca mulatta*	1/15/2019	4

Comments: None

AUTHORIZATION
VETERINARY PUBLIC HEALTH SECTION

Name: Curtis L. Fritz, DVM, PhD
Title: State Public Health Veterinarian
Date: 1/15/2019

Copies to: Importer or Agent ☑ Co. Environmental Health/County or PH Veterinarian ☑ Attending Veterinarian ☑

Veterinary Public Health Section, MS 7308, PO Box 997377, Sacramento, 95899-7377
Phone: 916.552.9740 Email: VetPH@cdph.ca.gov Fax: 916.552.9725

BH41

Date of birth: [redacted]
Sex: Male
Sire: [redacted]
Dam: [redacted]
Current Weight: 10.52 kg on 10/9/18

Rearing History:

- Rejected by birth mother. Cross-fostered to [redacted] and raised by cross-fostered mom until weaning at 8 months of age. Weaned into a peer group and socially housed until 6 years of age. Individually housed thereafter.

Experimental History:

- [redacted]

Breeding History:

- Used as a breeder male in large social pens.

Medical History:

[redacted]

TB test. Tested with mammalian tuberculin (0.1 mls ID) in the right eyelid.

					24 hr check	48 hr check	72 hr check
ID	Sex	Room	Cage	Date TB Test	Thurs 11/15	Fri 11/16	Sat 11/17
[redacted]							
BH41	M	41	23	11/14/18	–	–	–
[redacted]							

[redacted]

W-PRIMATE LAB
ENTERED

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN 53715

[redacted]

November 26, 2018

HEALTH STATEMENT

The rhesus monkeys (*Macaca mulatta*) listed below were examined by me on November 26, 2018 and found to be free of signs of contagious or communicable diseases. The last tuberculosis test was given as listed below, and the animals were non-reactive at 24, 48, and 72 hours. The antigen used was Old Tuberculin, 25 mg, given intradermally in the eyelid. These monkeys are not maintained on Isoniazid.

ID	SEX	DOB	Date of Last TB Test	ID	SEX	DOB	Date Of Last TB Test
[redacted]	M	5/13/95	11/14/18	BH41	M	8/6/11	11/14/18
[redacted]							

Lisa Krugner-Higby

Lisa Krugner-Higby, D.V.M.
Research Animal Veterinarian

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN 53715

[redacted]

November 26, 2018

To Whom It May Concern:

The rhesus monkeys (*Macaca mulatta)* in these cages are consigned by the following:

[redacted]

Lisa Krugner-Higby

Lisa Krugner-Higby, D.V.M.
Research Animal Veterinarian

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN 53715

[redacted]

November 26, 2018

CERTIFICATE OF ACCLIMATION

The rhesus monkeys (*Macaca mulatta)* in this shipment can adjust to temperatures as low as 40° F for periods not exceeding one hour. Temperatures lower than 40°F for periods longer than 1 hour may result in frostbite. Temperatures higher than 85°F must be avoided.

FOOD AND WATER INSTRUCTIONS

As these rhesus monkeys (*Macaca mulatta)* were fed and watered prior to delivery to the carrier and as succulent fruit has been provided in the cage, it is not necessary to provide food during the first 24 hours following delivery to the carrier. If shipment is delayed, food should be provided at least once every 24 hours. Water should be provided at least every 12 hours. Acceptable food items include monkey chow, dried fruits, apples, oranges, and bananas.

The rhesus monkeys (*Macaca mulatta)* in this shipment were offered water *ad lib* during the twenty four hour period prior to being picked up by the carrier. This animal has monkey chow available in the transport cage and had water available until right before shipment on the morning of departure. Succulent fruits were given to the animal in its cage to preclude thirst.

If necessary, information concerning feeding and watering may be obtained from the consignor at [redacted]

Lisa Krugner-Higby

Lisa Krugner-Higby, D.V.M.
Research Animal Veterinarian

UCLA
Division of Laboratory Animal Medicine

Animal Receiving Record

[redacted]

Date:	10-18-18	UCLA Protocol Number:	[redacted]	Experimental [X]
		Protocol Exp. Date:	5-15-2019 5-21-2019	UCLA Recharge ID Number: [redacted] 2 old animals; [redacted] 2 young animals. Please costs between these two fund numbers.

UCLA INFORMATION		OTHER INSTITUTION'S INFORMATION	
Institution:	*UCLA*	Institution:	[redacted]
Investigator:	[redacted]	Investigator:	
Laboratory Contact:		Laboratory Contact:	
Phone:		Phone:	
E-mail:		E-mail:	
Veterinarian:		Veterinarian:	
Phone:		Phone:	
E-mail:		E-mail:	
Import Coordinator:		Export Coordinator:	
Phone:		Phone:	
E-mail:		E-mail:	

Importing Animal Information		Shipping Charges	
Species:	Rhesus macaque	Who will pay for shipping?	[X] UCLA [] Sending Inst.
Number Receiving:	4	Courier	
#M / #F:	M	Account #	
Background Strain:	Indian origin rhesus	Comments:	
Genotype:			
Age:	21/23/6/7		

Immune status of the animals (*select only one*): [] Normal [] Deficient [] Undetermined

Have the animal(s) been inoculated and/or exposed to infectious agents, recombinant DNA, carcinogens, toxic chemicals, and/or radionucleotides? [] Yes [X] No

- If 'Yes' please identify:

Have the animal(s) had surgery or other experimental procedures performed on them? [X] No

Type of genetic modification: [] Tg [] KO [] KI [] N/A [] Other:

Is this a newly created genetically modified line? [X] No

Is a Material Transfer Agreement (MTA) required? [X] No

Source: [X] Domestic

Final *UCLA* Destination:	Facility	[redacted]	Room Number	[redacted]

Do the animals have any special housing or husbandry needs? [X] No

- *If 'YES' please explain on a separate sheet of paper*

Will you euthanize ALL animals within 12 hours of arrival? [X] No

Please provide the completed 'Animal Shipping Record' three (3) weeks prior to the shipping date

Revised: 10/26/17

[redacted]

California Department of Public Health CDPH

CDPH Use Only

Permit No. _____

APPLICATION FOR PERMIT TO IMPORT SPECIFIED WILD ANIMALS INTO CALIFORNIA

The CDPH Veterinary Public Health Section (VPHS) will endeavor to process permit applications in as timely a manner as possible; however, VPHS cannot guarantee that a permit will be issued by a specific date. Importers should submit permit applications to VPHS as soon as practically possible. The application form should be submitted to VPHS no less than ten (10) business days and all supplementary documents no less than five (5) business days preceding the anticipated date of shipment. Please submit all application documents by email to VetPH@cdph.ca.gov.

1. A permit is requested to import the following specified wild animals, in accordance with the provisions of the California Code of Regulations (CCR), Title 17, § 30070 - 30086.

Quantity	Common Name *	Scientific Name
4	Rhesus Macaque	Macaca Mulatta

* Mammals of the World. Walker E.P., et. al.; Johns Hopkins Press, Baltimore.

2. Are the above animals considered California Department of Fish and Wildlife (CDFW) Restricted Species (14 CCR § 671)? ☑ No ☐ Yes

If Yes, please provide the applicable CDFW Permit No(s). and Expiration Date(s)_____

3. Purpose of importation: ☐ Re-Entry ☑ Research ☐ Zoological Exhibition ☐ Non-Zoo Exhibition ☐ Short-term Exhibition (No Contact) ☐ Short-term Exhibition (Human Contact)

4. Expected point of first arrival in California: UCLA Anticipated arrival date: 11/29/2018

5. Name of carrier: [redacted] Transportation mode: Truck

6. Place where animal(s) will be quarantined: UCLA

	Name	Address	Telephone	Email Address	Accreditation
Consignor (origination)	[redacted]				☐ AZA ☐ AAALAC
Consignee (destination)					☐ AZA ☑ AAALAC
Veterinarian					

7. Amount Due: 40.00 ($10.00 per animal: Send check or money order payable to the California Department of Public Health)

DDI

UCLA
Division of Laboratory Animal Medicine

Mouse Incoming Animal Record

[x] STANDARD USE

Deliver to: [x] NHP Quarantine

Quar. Rm#: [redacted]

Final Facility: [redacted]

DLAM Import Coordinator to Complete

Approval Date: November 29th, 2018	Sending Facility Information	UCLA Information (Receiving Facility)
Breed/Strain: Rhesus Macaque	Facility: University of Wisconsin	PI: [redacted]
# of Animals: 4	PI: [redacted]	Prot.#: [redacted] [x] Experimental
4 Males: [redacted] BH41 (8/6/11), [redacted]	Veterinarian: [redacted] Phone: [redacted] Email:	Prot. Expiration Date: 5/15/19 / 5/21/19
UCLA Veterinarian: [redacted]	Sending Facility Contact: [redacted] Phone: Email: [redacted]	Recharge ID #: [redacted]
		Lab Contact: [redacted] Phone: Email:

Courier Used: [redacted] Waybill #: [redacted] Notes: [redacted]

[x] Health Status Information received and incoming mice approved for DLAM housing: [redacted]
DLAM Import Coordinator

DLAM Technician to Complete

Date Received: 11/30/18 Animal Condition: [✓] Good [ ] *Poor

Total # Received: 4 # Males 4 # Females ____ Total # of cages set up: 4 ech. Initials: [redacted]

(*Notify Import Coordinator if receipt condition is Poor)

Ship Date: November 28th, 2018

COPY

This record is required by law (7 USC 2131-2156). (9 CFR Subchapter A, Parts 1, 2 and 3) Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 y ear, or a fine of not more than $1 000, or both.

See reverse side for additional information

FORM APPROVED
OMB NO. 0579-0036

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

RECORD OF ACQUISITION, DISPOSITION OR TRANSPORT OF ANIMALS (Other Than Dogs and Cats)

☒ SALE ☐ EXCHANGE OR TRANSFER ☐ DONATION

1. INVOICE NO.	2. PAGE
	1 of 1

3. DATE OF DISPOSITION: 11/28/18

4. DEALER'S LICENSE NO.: [redacted]

INSTRUCTIONS: Complete applicable items 1 through 13. Original and one copy to accompany animals. When delivery is made - items 14 through 20 must be completed. Original retained by Buyer (Receiver) and copy one returned to Dealer (Seller or Donor). Copy two to be retained by Dealer (Seller or Donor). Attach Continuation Sheet (APHIS FORM 2070A) as needed.

5. SELLER OR DONOR (Name and Address, Include Zip Code): [redacted]

6. BUYER OR RECEIVER (Name and Address, Include Zip Code): [redacted]

7. USDA LICENSE NO. (If any):

8. IDENTIFICATION OF ANIMALS BEING DELIVERED

A. CONTAINER TAG NO. CRATE OR PEN NO.	B. NO ANIMALS	C. PREVIOUS INVOICE NO. (If any)	D. INDIVIDUAL IDENT TATTOOS, TAG NOS. (If applicable)	E. SPECIES	AGE - SEX F. NO. YOUNG	AGE - SEX G. NO. ADULT	H. EST. WEIGHT (lbs.)	I. REMARKS (Condition, etc.)	RECEIVER'S USE J.	RECEIVER'S USE K.
	1		[redacted]							
	1									
	1		BH41	rhesus monkey	M F	M X F	22.1	Healthy		
	1		[redacted]							
					M F	M F				
					M F	M F				
					M F	M F				
					M F	M F				
					M F	M F				
					M F	M F				
					M F	M F				

DELIVERY BY COMMERICAL CARRIER

9. DELIVERY BY ("X" one)	10. TRUCK LICENSE NO.	11. BILL OF LADING NO.

12. NAME AND ADDRESS OF COMPANY OR FIRM

13. NAME AND ADDRESS OF TRUCK DRIVER

COPY

DELIVERY RECEIPT - TO BE COMPLETED BY BUYER OR RECEIVER

14. ANIMALS DELIVERY WERE ("X" ONE)

☐ IN APPARENT GOOD CONDITION ☐ POOR CONDITION ☐ REJECTED (Attach explanation for rejection)

15. TOTAL NUMBER RECEIVED	16. NUMBER DEAD	17. NUMBER ALIVE

18. BY (Signature)	19. TITLE	20. DATE

APHIS FORM 7020 (APR 1993)

AH-IN-20 (rev 11/2012)

COPY

Page 1 of 1

35 - 204583

WISCONSIN INTERSTATE SMALL ANIMAL CERTIFICATE OF VETERINARY INSPECTION

Ch. ATCP 10, Wis. Admin. Code; Ch. 95, Wis. Stats.

PLEASE PRINT LEGIBLY

SUBMIT ORIGINAL WITHIN 7 DAYS AFTER ISSUE TO:
Department of Agriculture, Trade and Consumer Protection
Division of Animal Health
P.O. Box 8911, Madison, WI 53708-8911
Phone: 608-224-4872 Fax: 608-224-4871

TYPE OF ANIMAL SHIPPED
☐ Dog ☐ Cat ☒ Non-human Primate
☐ Other: _____

PERMIT NUMBER (If applicable)

SHIPMENT
☐ Returning to WI
☒ Not returning to WI
Number of Animals in Shipment: 4
Shipping date: 11-28-18

Owner or Consignor [redacted]
Origin Street Address
Origin City / State / Zip
Owner Mailing Address / City / State / Zip (If different than above)

Consignee or Destination [redacted]
Destination Street Address
Destination City / State / Zip
Destination Mailing Address / City / State / Zip (If different than above)

☐ Animals are traveling with owner on vacation

	Breed	Individual Identification (Name, Description of Markings, Microchip, etc.)	Sex	Age	Rabies Vaccination Date	Rabies Vaccination Exp. Date	Product & Vaccine Producer	Serial Number	Rabies Tag Number	Other Vaccinations	Date Vaccinated	Product & Vaccine Producer
1	[redacted]											
2												
3	Rhesus	BH41	M	7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
4	[redacted]											
5												
6												
7												

VETERINARIAN: I certify as a veterinarian, accredited and certified by the State of Wisconsin, that the described animal(s) have been inspected by me and that they are not showing any signs of infectious, contagious and/or communicable disease (except where noted). The vaccinations and results of tests are as indicated on this certificate. To the best of my knowledge, the animal(s) listed on this certificate meet the state of destination and Federal interstate requirements. No warranty is made or implied.

OWNER / AGENT STATEMENT: I certify the animal(s) in this shipment are as listed on this certificate. [redacted]

[redacted]

[redacted] information you provide may be used for purposes other than that for which it was originally collected – sec. 15.04(1)(m), Wis. Stats. [redacted] Equal Opportunity Employer

WHITE (WI State Veterinarian), CANARY (State Veterinarian of destination), PINK (accompany shipment), GOLDENROD (retained by issuing veterinarian)

ANIMAL HEALTH OBSERVATION LOG

(White Copy: JKL Secure Freight – Yellow Copy: Receiving Facility – Pink Copy: Shipper)

DATE: 11-28-18 [redacted] SHIPMENT NUMBER: [redacted]

QUANTITY OF CRATES RECEIVED:	4	QUANTITY OF ANIMALS RECEIVED:	4
TYPE OF FOOD PROVIDED TO ANIMALS:	Apples / Biscuits		
GENERATOR(S), A/C, AND HEATING UNIT(S) TESTED AND FUNCTION PROPERLY; CARGO AREA SANITIZED (INITIAL & DATE):	11-28-18		

TIME (In PST zone)	ANIMAL QUANTITY	OBSERVATIONS, TREATMENTS, AND / OR ACTIONS TAKEN	CARGO AREA TEMP (°F)	INITIAL & DATE
7:00 a.m. (p.m.)	4	All Active & Alert Picked up at University Wisconsin NPRC Feed By NPRC water By JKL	70°	[redacted] 11-28-18
8:00 a.m. 810 p.m.	4	All Active & Alert Belmont WI	73°	[redacted] 11/28/18
1159 a.m. 1210 p.m.	4	All Active And Alert marengo IA	74°	[redacted] 11/28/18
358 a.m. 414 p.m.	4	All Active And Alert water given Beaver crossing NE	76°	[redacted] 11/28/18
7:57 a.m. 8:02 p.m.	4	All Active & Alert OGallala NE	70°	[redacted] 11-28-18
11:56 a.m. 12:02 p.m.	4	All Active & Alert Colorado	73°	[redacted] 11-28-18
3:55 a.m. 4:15 p.m.	4	All Active & Alert Feed & Water given Gypsum CO	71°	[redacted] 11-29-18
7:54 a.m. 8:00 p.m.	4	All Active & Alert Emee County UTAH	73° ~~Hot 18~~	[redacted] 11-29-18
1153 a.m. 1210 p.m.	4	All Active And Alert New Harmony UT	75°	[redacted] 11/29/18
352 a.m. 410 p.m.	4	All Active And Alert Baker CA Water given	69°	[redacted] 11/29/18
750 a.m. p.m.	4	All Active And Alert Drop off cargo at UCLA	71°	[redacted] 11/29/18
a.m. p.m.				
a.m. p.m.				

COPY

4 (QUANTITY) ANIMALS WERE RECEIVED BY UCLA (PRINTED COMPANY NAME) IN SATISFACTORY CONDITION. [redacted]

NAME OF PERSON RECEIVING ANIMALS [redacted] POSITION [redacted] 11/29/18

RECEIVING COMPANY REPRESENTATIVE INITIAL & DATE

UCLA
Division of Laboratory Animal Medicine

Species	Temp. Set Pt. °F (°C)	Temp. Range °F (°C)	Humidity Range
NHP	74° (23°)	64 – 84° (18 -29°)	30 – 70%

NHP Room Status Sheet

Species: NHP | **Month:** Jan | **Year:** 20 | **Facility:** [redacted] | **Room #** [redacted]

	Environment - Daily			Husbandry Twice Daily				Husbandry - Daily				
Date	Temp.	Humidity	Air Flow +/-	AM Animal Health √ (time)	AM Feed/ water √ (time)	PM Feed/ water √ (time)	Change Cage Pan √	Sanitize Floors √	Empty Trash √	*BRI Enrichment Frig. Temp. (F)	Initials
1	72.1	47.4	—	√700	√700	12:10 [redacted]	✓	✓	✓		[redacted]
2	72.3	57.0	—	√800	√800	130 ✓	✓	✓	✓		
3	72.3	47.4	—	√700	√700	130 [redacted]	✓	✓	✓		
4	72.4	46.8	—	√8:20	√8:20	12:10PM [redacted]	✓	✓	✓		
5	73.1	48.7	—	√8:20	√8:20	11:45 [redacted]	✓	✓	✓		
6	72.3	39.8	—	√630	√630	200 PM ✓	✓	✓	✓		
7	72.3	40.3	—	√600	√600	√130	✓	✓	✓		
8	72.1	61.8	—	√630	√630	200 ✓ [redacted]	✓	✓	✓		
9	72.8	50.6	—	√705	√ur	12 00 [redacted]	✓	✓	✓		
10	72.5	44.9	—	√630	√630	000 [redacted] ✓	✓	✓	✓		
11	72.0	53.5	—	√700 [redacted]	√700 [redacted]	11:35 [redacted]	✓	✓	✓		
12	72.5	46.9	—	√760 [redacted]	√700 [redacted]	1130 [redacted]	✓	✓	✓		
13	72.3	58.0	—	√600 [redacted]	√600 [redacted]	130 [redacted]	✓	✓	✓		
14	72 3	62.2	—	√700	√700	200 [redacted]	✓	✓	✓		
15	72.3	48.0	—	√630	√630	130 [redacted]	✓	✓	✓		
16	72.3	60.1	—	√630	√630	130 [redacted]	✓	✓	✓		
17	72.3	50.0	—	√630	√630	100 [redacted]	✓	✓	✓		
18	72.3	49.2	—	√6:35	√6:35	√1135 [redacted]	✓	✓	✓		
19	72-3	37-6	—	√6:30.1	√6:30	√12:00 [redacted]	✓	✓	✓		
20	72 3	60.4	—	√6:15	√6:15	√12:10 [redacted]	✓	✓	✓		
21	72.3	45.3	—	√630 [redacted]	√630 [redacted]	√130 [redacted]	✓	✓	✓		
22	73 0	64.2	—	√630	√630	100 [redacted]	✓	✓	✓		
23	72.5	54.2	—	√630	√630	100 [redacted]	✓	✓	✓		
24	72-3	51.6	—	√630	√630	1100 [redacted]	✓	✓	✓		
25	72.3	52.2	—	√6.00	√6:00	12:00 [redacted]	✓	✓	✓		
26	72.3	51.8	—	√7:00	√7:00	1:00 ✓	✓	✓	✓		
27	72.1	49.8	—	√630	√630	130	✓	✓	✓		
28	72.3	45.7	—	√630	√630	130 ✓	✓	✓	✓		
29	72.1	46.4	—	√630	√630	130 ✓	✓	✓	✓		
30	73.3	37.9	—	√630	√630	130 ✓	✓	✓	✓		
31	72.3	45.9	—	√700	√760	100 [redacted]	✓	✓	✓		

*Temp is not recorded on weekends or holidays

[redacted] 2/3/20

UCLA
Division of Laboratory Animal Medicine

Species	Temp. Set Pt. °F (°C)	Temp. Range °F (°C)	Humidity Range
NHP	74° (23°)	64 – 84° (18 -29°)	30 – 70%

NHP Room Status Sheet

Species: NHP | **Month:** JAN | **Year:** 2020 | **Facility:** [redacted] | **Room #:** [redacted]

	Environment - Daily			Husbandry Twice Daily			Husbandry - Daily				
Date	Temp.	Humidity	Air Flow + / -	AM Animal Health √ (time)	AM Feed/ water √ (time)	PM Feed/ water √ (time)	Change Cage Pan √	Sanitize Floors √	Empty Trash √	*BRI Enrichment Frig. Temp. (F)	Initials
1	71.1	45.5	—	√700 [redacted]	√700 [redacted]	12:30 PM	✓	✓	✓	N/A	[redacted]
2	72.0	66.6	—	√800 [redacted]	√800	1:30pm	✓	✓	✓		
3	70.0	48.4	—	√700	√700	130 [redacted]	✓	✓	✓		
4	70.4	47.4	—	✓8:30	✓8:30	12:30 [redacted]	✓	✓	✓		
5	71.3	46.5	—	✓8:40	✓8:40	12:00 [redacted]	✓	✓	✓		
6	71.8	46.6	—	√630	√630	200pm	✓	✓	✓		
7	72.0	39.9	—	√600	√600	1:30	✓	✓	✓		
8	72.0	64.4	—	√630	√630	√200	✓	✓	✓		
9	72.0	52.6	—	710	✓7[redacted]	12 00	✓	✓	✓		
10	72.1	45.4	—	√630	√630	100 [redacted]	✓	✓	✓		
11	72.0	53.3	—	700 ✓	√700	1135 [redacted]	✓	✓	✓		
12	72.1	49.1	—	√700 [redacted]	√700 [redacted]	1130 [redacted]	✓	✓	✓		
13	72.0	60.4	—	√600 [redacted]	√600 [redacted]	130 [redacted]	✓	✓	✓		
14	72.0	60.6	—	√700 [redacted]	√700 [redacted]	200 [redacted]	✓	✓	✓		
15	72.0	43.7	—	√630 [redacted]	√630 [redacted]	130 [redacted]	✓	✓	✓		
16	72.0	61.1	—	√630 [redacted]	√630 [redacted]	130 [redacted]	✓	✓	✓		
17	72.0	54.9	—	√630 [redacted]	√630 [redacted]	100 [redacted]	✓	✓	✓		
18	72.0	41.8	—	√7:00 [redacted]	√7:00 [redacted]	1CRT [redacted]	✓	✓	✓		
19	72.0	38.3	—	√7:00 [redacted]	√7:00 [redacted]	12:10 [redacted]	✓	✓	✓		
20	71.8	64.3	—	√6:55 [redacted]	√6:55 [redacted]	12:30 [redacted]	✓	✓	✓		
21	72.0	46.6	—	√630 [redacted]	√630 [redacted]	130 [redacted]	✓	✓	✓		
22	71.8	64.3	—	√630 [redacted]	√630 [redacted]	100 [redacted]	✓	✓	✓		
23	72.1	58.2	—	√630 [redacted]	√630 [redacted]	100 [redacted]	✓	✓	✓		
24	72.1	54.6	—	√630 [redacted]	√630 [redacted]	100 [redacted]	✓	✓	✓		
25	72.3	53.8	—	√6:30 [redacted]	√6:30 [redacted]	12:40 [redacted]	✓	✓	✓		
26	72.0	53.6	—	√7:30 [redacted]	√7:30 [redacted]	1:08 [redacted]	✓	✓	✓		
27	71.0	47.9	—	√630 [redacted]	√630 [redacted]	130 [redacted]	✓	✓	✓		
28	71.8	46.9	—	√630 [redacted]	√630 [redacted]	130 [redacted]	✓	✓	✓		
29	72.0	48.2	—	√630 [redacted]	√630 [redacted]	130 [redacted]	✓	✓	✓		
30	72.0	39.4	—	√630 [redacted]	√630 [redacted]	130 [redacted]	✓	✓	✓	N/A	
31	71.8	47.9	—	√700 [redacted]	√700 [redacted]	100 [redacted]	✓	✓	✓		

*Temp is not recorded on weekends or holidays

[redacted] 2/3/20

UCLA
Division of Laboratory Animal Medicine

Species	Temp. Set Pt. °F (°C)	Temp. Range °F (°C)	Humidity Range
NHP	74° (23°)	64 – 84° (18 -29°)	30 – 70%

NHP Room Status Sheet

Species: NHP | **Month:** 2-1-2020 **Year:** | **Facility:** [redacted] | **Room #** [redacted]

	Environment - Daily				Husbandry Twice Daily			Husbandry - Daily			
Date	Temp.	Humidity	Air Flow +/-	AM Animal Health √ (time)	AM Feed/ water √ (time)	PM Feed/ water √ (time)	Change Cage Pan √	Sanitize Floors √	Empty Trash √	*BRI Enrichment Frig. Temp. (F)	Initials
1	72.3	44-7	—	✓6:00	✓6:00	11:40	✓	N/A	✓	N/A	[redacted]
2	72-5	48-7	—	✓6:00	✓6:00	✓11:30	✓	N/A	✓	N/A	
3	72.1	45.4	—	✓630	✓630	130	✓	✓	✓		
4	72.5	26.0	—	✓630	✓630	130	✓	✓	✓		
5	72.3	39.9	—	✓630	✓630	130	✓	✓	✓		
6	72.1	43.9	—	✓630	✓630	130	✓	✓	✓		
7	72.3	50.4	—	✓630	✓630	130	✓	✓	✓		
8	72.3	57.2	—	✓630	✓630	1130	✓	✓	✓		
9	72.3	49.7	—	✓630	✓630	11:45	✓	✓	✓		
10	72.3	45.2	—	✓630	✓630	200	✓	✓	✓		
11	72.3	37.6	—	✓630	✓630	130	✓	✓	✓		
12	72.1	51.3	—	✓630	✓630	130	✓	✓	✓		
13	72.1	52.7	—	✓6:50am	✓6:50am	✓1:30pm	✓	✓	✓		
14	72.5	50.5	—	✓7:30am	✓7:30am	✓1:30pm	✓	✓	✓		
15	72.5	51.7	—	✓7:25am	✓7:25am	12:30 pm	✓	✓	✓		
16	72.5	57-6	—	✓7:10 AM	✓7:10 AM	11:55 AM	✓	✓	✓		
17	72.3	51.0	—	✓6:30 AM	✓6:30 AM	11:55 AM	✓	✓	✓		
18	72.5	53.8		✓630 [redacted]	✓630 [redacted]	130 [redacted]	✓	✓	✓		
19	72.5	48.0	—	✓630	✓630	130	✓	✓	✓		
20	72.0	48.3	—	✓630	✓630	130	✓	✓	✓		
21	72.3	50.4	—	600 ✓ [redacted]	✓600	130 [redacted] ✓	✓	✓	✓		
22	72.5	49.8	—	6:30 Am	6:30 Am	1140 [redacted] ✓	✓	✓	✓		
23	72.5	58.6	—	6:20 am	6:20 Am	1135	✓	✓	✓		
24	72.5	58.7	—	630 [redacted]	630 [redacted]	130	✓	✓	✓		
25	72.5	52.2	—	✓630	✓630	130	✓	✓	✓		
26	72.3	40.8	—	✓600	✓600	130	✓	✓	✓		
27	72.5	37.0	—	✓630	✓630	130	✓	✓	✓		
28	72.3	40.1	—	✓630	✓630	130	✓	✓	✓		
29	72.3	55.7	—	✓630	✓630	1130 [redacted]	✓	✓	✓		
30											
31										N/A	

*Temp is not recor [redacted]

Rev.07/2018

Form#242(A) Page 1 of 2

UCLA
Division of Laboratory Animal Medicine

Species	Temp. Set Pt. °F (°C)	Temp. Range °F (°C)	Humidity Range
NHP	74° (23°)	64 – 84° (18 -29°)	30 – 70%

NHP Room Status Sheet

Species: NHP | **Month:** FEB 1-2020 | **Year:** | **Facility** [redacted] | **Room** [redacted]

	Environment - Daily			Husbandry Twice Daily			Husbandry - Daily				
Date	Temp.	Humidity	Air Flow +/-	AM Animal Health √ (time)	AM Feed/ water √ (time)	PM Feed/ water √ (time)	Change Cage Pan √	Sanitize Floors √	Empty Trash √	*BRI Enrichment Frig. Temp. (F)	Initials
1	72.0	49.1	—	√ 6:25	√ 6:26	√ 11:55	√	N/A	√	N/A	[redacted]
2	72.1	51.2	—	√ 6:30	√ 6:30	√ 11:40	√	N/A	√	N/A	
3	72.0	39.0	—	√ 630	√ 630	130	√	√	√	N/A	
4	72.0	26.1	—	√ 630	√ 630	130	√	√	√		
5	71.8	32.8	—	√ 630	√ 630	130	√	√	√		
6	72.0	45.4	—	√ 630	√ 630	130	√	√	√		
7	72.0	53.2	—	√ 630	√ 630	130	√	√	√		
8	72.0	58.5	—	√ 630 [redacted]	√ 630 [redacted]	11 30 [redacted]	√	√	√		
9	72.1	48.2	—	√ 630	√ 630	11 45	√	√	√		
10	72.1	42.1	—	√ 630	√ 630	100 [redacted]	√	√	√		
11	72.0	38.2	—	√ 630	√ 630	130 [redacted]	√	√	√		
12	71.8	61.7	—	√ 630	√ 630	130	√	√	√		
13	72.1	53.5	—	√ 6:50 [redacted]	√ 6:50 am	√ 1:30 pm	√	√	√		
14	72.1	52.2	—	√ 7:30 am	√ 7:31 am	√ 1:30 pm	√	√	√		
15	72.0	53.5	—	√ 7:25 am	√ 7:25 am	√ 12:15 pm	√	√	√		
16	72.0	59.0		√ 6:50 AM	√ 6:50 AM	11:40 PM	√	√	√		
17	72.0	53.2	—	√ 7:10 AM	√ 7:00 AM	12:00 PM	√	√	√		
18	72.1	52.1	—	√ 630 [redacted]	√ 630 AM	130 [redacted]	√	√	√		
19	72.1	48.2	—	√ 630 [redacted]	√ 630 [redacted]	130 [redacted]	√	√	√		
20	72.0	49.5	—	√ 630 [redacted] AM	√ 630 [redacted]	130 [redacted]	√	√	√		
21	72.0	50.0		√ 606 AM	√ 606 AM	130 [redacted]	√	√	√		
22	72.0	50.5	—	√ 7:10 AM	√ 7:10 AM	11 50 [redacted]	√	√	√		
23	72.1	61.1	—	√ 6:55 PM	√ 6:55 PM	1140 [redacted]	√	√	√		
24	72.0	63.9	—	√ 630 [redacted]	√ 630 [redacted]	130 [redacted]	√	√	√		
25	71.8	53.5	—	√ 630	√ 630	130 [redacted]	√	√	√		
26	72.1	43.3		√ 600	√ 600	130 [redacted]	√	√	√		
27	72.1	44.2	—	√ 630	√ 630	130 [redacted]	√	√	√		
28	71.8	41.6	—	√ 630	√ 630	130 [redacted]	√	√	√		
29	76.0	52.5	—	√ 630	√ 630	11 30 [redacted]	√	√	√		
30										N/A	
31											

*Temp is not recorded on w[redacted]

2/2/20

Enrichment Log (one sheet per animal)	MONTH:	January	FACILITY:	[redacted]	TECH(S):	[redacted]
SPECIES: PRIMATE	YEAR:	2020	ROOM:	[redacted]	ANIMAL ID:	BH41 [redacted]

Devices/Toys – Provided per DLAM species-specific SOP, protocol or veterinary discretion. Record number under date from 'key' at bottom of page.
Record items not listed next to "other" and initial.

Device/Toy ↓ Date:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	17	17	17	17	17	17	17	17							1	1	1	1	1	1	1	1									
	2	2	2	2	2	2	2	2							2	2	2	2	2	2	2	2									
	20	20	20	20	20	20	20	20	3	3	3	3	3	3	17	17	17	17	17	17	17	17	12	12	12	12	12	12	12	3	3
									13	13	13	13	13	13									16	16	16	16	16	16	16	13	13
									20	20	20	20	20	20									20	20	20	20	20	20	20	20	20
Other:																															
Other:																															
Other:																															

Food Treats- Provided per DLAM species-specific SOP, protocol or veterinary discretion.
Record 'key' treat item in space provided and initial under date. List items not listed in menu 'key' as other.

Treat ↓ Date:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Menu Item (wet)	[redacted]																														
Menu Item (dry)																															
Other:																															
Other:																															
Other:																															
Other:																															
Other:																															
Other:																															
Other:																															

Additional/Supplemental Enrichment – Record additional supplement and initial under date.

Supplement ↓ Date:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Forage Mix	[redacted]																														
Other: TV TIME																															
Other:																															
Other:																															
Other:																															

1- Mirror 2- Keys 3- Ball 4- Shelter/Hide 5- Dental Chew 6- Molecule 7- Traffic Cone 8- Porci-Chew 9- Wood 10- PVC Pipe 11- Rattle
12- Kong 13- Tire Biter 14- Challenger Ball 15- Puzzle Feeder 16- Barbell 17- Havaball 18- Maracas 19- Bell 20- Chains 21- Bones 22- Spiral feeder

[redacted]

MINUTES
Chancellor's Animal Research Committee
Monday, May 4, 2020

Meeting Start Time: 2:30 pm
Meeting End Time: 3:45 pm
Meeting Chaired by: Nigel Maidment
Minutes Prepared by: ARC Administrative office [redacted]

Members in Attendance (13 voting, 1 alternate)
[redacted] (Scientist)
[redacted] (Non-affiliate, Community Member, Non-Scientist)
[redacted] (Scientist)
[redacted] (Non-affiliate, Community Member, Non-Scientist)
[redacted] (Vice-Chair, Scientist)
Jeffrey Goodwin (Attending Veterinarian)
[redacted] (Member)
[redacted] (Vice-Chair, Scientist)
Nigel Maidment (Chair, Scientist)
[redacted] (Scientist)
[redacted] (Scientist)
[redacted] (Scientist)
[redacted] (DLAM Veterinarian, Alternate Member for Jeff Goodwin)[1]
[redacted] (Scientist)

Members Absent (2 voting, 1 alternate):
[redacted] (Scientist)
[redacted] (DLAM Veterinarian, Alternate Member for Jeff Goodwin)
[redacted] (Scientist)

Nonmembers in Attendance:
[redacted] (ARC Coordinator)
[redacted] (ARC Coordinator)
[redacted] (ARC Administrator)
[redacted] (RSAWA Director)
[redacted] (ARC Coordinator)

The meeting was called to order at 2:30 pm.

Due to the COVID19 pandemic, this meeting was conducted virtually via Zoom.

[1] As alternate for Dr. Goodwin, [redacted] participated in the meeting discussions, but did not vote.

1. Review of April 20, 2020 ARC Meeting Minutes

A motion to approve the April 20, 2020 meeting minutes passed unanimously: 12 yes, 0 no, 0 abstain.

One voting member joined the call after the vote, increasing the number of voting members to 13.

2. Review of Applications

New (1)
ARC [redacted] (Species: Pig)

Renewals (3)
ARC [redacted] (Species: Mouse, Rat)

ARC [redacted] (Species: Mouse) – The members discussed the potential for the animals to experience more than momentary pain or discomfort in the context of the potential for gaining knowledge that may benefit human or animal health. Information gained from understanding the cellular function in regulating proinflammatory response during colitis may help the lab develop therapeutic interventions for inflammatory bowel disease. As such, the potential benefits to human or animal health were considered strong justification for the use of animals in the research, including animals in Pain Category E.

[redacted] *left the call at 3PM; however, this did not impact quorum.*

Amendment (2)
ARC [redacted] (Species: Chipmunk, Squirrel)

Continuation (1)
ARC #[redacted] (Species: Monkey) – [redacted] *and* [redacted] *left during this discussion due to a conflict of interest*. The Vice Chair provided a brief summary of the research and informed the members of a reported adverse event. The description of the adverse event included information regarding pending histological analyses that, once received, will inform potential refinements to the surgical procedure.

The AV reminded the members of the April 20, 2020 discussion where the PI detailed consultation with vets and NHP experts from other institutions on potential treatments for animal welfare related conditions. The AV reminded the members of the AV's responsibility when collaborating with investigators on treatment of their research animals, emphasizing the need for a collegial and collaborative approach. The ARC agreed that the AV, as mandated by the Animal Welfare Act and PHS Policy, and in the context of a professional and collegial relationship with the research community, should provide treatment recommendations for sick or injured animals to PIs as relevant based on medical expertise and differential diagnoses. PIs receiving these recommendations should consider them in the context of their experimental goals, with the final decision on the chosen treatment being designated to the AV. The ARC acknowledged that all parties seek to ensure high standards of animal welfare and care.

Unless otherwise specified in these minutes, further review of the above protocols will be conducted using DMR subsequent to FCR with review of PI responses to the ARC's questions and requests designated to the Chair or Vice-Chair in accordance with UCLA's Animal Welfare Assurance. A vote to proceed as discussed passed unanimously (13 yes, 0 no, 0 abstain, except for ARC [redacted] which passed 11 yes, 0 no, 0 abstain due to the aforementioned recusals).

3. Standard Procedures for New RATS

The members were asked to review and approve the following standard procedures for use in the new RATS program:

- Anesthesia, Terminal, Pentobarbital (Mouse, Rat, Macaque)
- Anesthesia, Terminal, Ketamine/Xylazine (Mouse, Rat)
- Anesthesia, Ketamine/Xylazine (Mouse, Rat)
- Anesthesia, isoflurane (under 1 hour) (Mouse, Rat, Hamster, Guinea Pig)
- Anesthesia, isoflurane (over 1 hour) (Mouse, Rat, Hamster, Guinea Pig)

The members determined that comments submitted by the vets and ARC Office will be incorporated into the procedures with final review by the DLAM vet and the Chair. A vote to approve the procedures via this process and proceed as discussed passed unanimously (13 yes, 0 no, 0 abstain).

4. Continuing Education for ARC Members

None

5. Chair/Vet/RSAWA News and Notes

a) STARS Compliance Follow-up, ARC [redacted] (Species: Mouse)
The members were reminded of their February 10, 2020 meeting at which they determined that a lab manager should meet with the ARC Administrator for a remote STARS meeting. At the current meeting, the ARC Administrator summarized the resulting April 23, 2020 meeting with the lab manager, reporting a collegial discussion that resulted in an agreed-upon action plan for better communication regarding DLAM health case emails. The members determined that an email communication will be sent to the PI indicating that the issue has been considered resolved. A vote to proceed as discussed passed unanimously (13 yes, 0 no, 0 abstain).

b) Edits to Standard Procedures in New RATS:
The RSAWA Director informed the members that, following discussion with the DLAM vets, edits were proposed to the Survival Surgery template in new RATS to address the use of specific sized clippers and provide an option to use hair removal cream. In addition, edits were proposed to include language regarding administration of pre-operative analgesia. A vote to approve these changes passed unanimously (13 yes, 0 no, 0 abstain).

c) ARC Policy on Restricted Access to Water:
The members were informed of an ARC member's request to review the ARC Policy for Restricted Access to Water in order to determine if revisions should be made to account for the required frequency of weight measurements. The members determined that a review of potential revisions to this policy will be added to the May 18, 2020 meetings agenda. No vote

With no additional committee business or questions, the meeting adjourned at 3:45 pm.

END OF MEETING MINUTES

Approved: May 18, 2020 [redacted]