From: [redacted]
To: APHIS-AnimalCare; Lowe, Mark; [redacted]
Cc: [redacted]
Subject: [External Email]Exemption Request
Date: Monday, September 26, 2022 10:28:12 AM
Attachments: USDA Exemption Request [redacted] 9.22.22-MELsigned.pdf
[redacted] ApprovalLetter_IACUC.pdf
[redacted] Approved IACUCForm_22-0184.pdf
Non-Human_Primate_Socialization_and_Environmental_Enrichment_Policy.pdf

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USDA/APHIS/AC –

On behalf of our Institutional Official, Dr. Mark Lowe, I have attached materials related to Washington University in St Louis' request for an exemption. Our USDA registration number is 43-R-0008 and our OLAW Assurance number is D16-00245 (previously A3381-01).

Please let me know if you have any questions or require any additional information.

[redacted]
Director, IACUC Office
[redacted]@wustl.edu
phone: [redacted]

Washington University in St. Louis

Interim Vice Chancellor for Research, Washington University
Associate Dean for Research, School of Medicine
Harvey R. Colten Professor of Pediatric Science

September 22, 2022

USDA/APHIS
Animal Care
4700 River Road, Unit 84
Riverdale, Maryland 20737-1234

Re: Exemption Request; 9 CFR, Part 2, Section 2.31 (d)(1)(x)(C)
IACUC Protocol number 22-0184 (unique identifier)

Dear Animal Care Regional Director and Animal Care Deputy Administrator –

I am writing to request an exemption to 9 CFR, Part 2, Section 2.31 (d)(1)(x)(C) concerning multiple survival surgery in covered species for two non-human primates (macaca mulatta). The IACUC, with the support of the Attending Veterinarian and the Director for Large Animal Health Services, has reviewed and approved the proposed exemption. All other stipulated requirements of the Animal Welfare Act and regulations will be met in consideration of this exemption.

The Washington University in St. Louis (WUSTL) has welcomed [redacted]. [redacted] research focus delves into how brain circuits organize incoming sensory data into meaningful collections of objects and background. Understanding these processes is an essential step towards tackling conditions, like schizophrenia, where this processing function is perturbed.

[redacted] plans to continue similar studies begun under his postdoctoral mentor, [redacted]. The work is currently funded by the NIH award "Border ownership and grouping in primate visual cortex (EY031795)". A full description of the proposed WUSTL studies is included in the attached PDF copy of the IACUC approved protocol. This document includes the type and number of procedures, the frequency of procedures, and the time periods between procedures. Measures to minimize pain and distress are detailed in the attached protocol (anesthesia, analgesics) and the attached IACUC Policy "Nonhuman Primate Socialization and Environmental Enrichment".

Procedural summaries and scientific justifications for each exemption are listed below. Non-human primates are permanently and uniquely identified using individual tattoos. However, the proposed research participation plans and scientific justifications will be presented using the assigned names.

[redacted] has received one major survival surgery for a headpost implantation. Although this procedure was performed under [redacted]' protocol at the [redacted], this is the same initial procedure required for [redacted] research program and the existing headpost can be utilized for his continued studies at WUSTL. Studying the neural activity underlying the segmentation of visual scenes using laminar multielectrodes or multiphoton imaging requires installing a head post to obtain the required stability during the recording. [redacted] would continue to

Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8106-0016-10, St. Louis, Missouri 63110-1093
Phone: (314) 747-0515, lowe@wustl.edu

participate in research as described in the approved WUSTL protocol. The timeframe would be no more than 15 years of service or the humane endpoint, whichever is earlier. Total years of service would be calculated as the sum of research participation at [redacted] and WUSTL.

Justification: As described in the protocol, [redacted] research program requires nonhuman primates because
(1) all of the prior work upon which the program is based used the macaque;
(2) the phylogenetic proximity of Old World monkeys to humans and close similarities of their brains to the human brain, especially for the visual system, make the results relevant to an understanding of human vision and cognition, and to the development of clinical procedures for treatment of disorders of these systems; and
(3) the macaque is capable of performing complex tasks, including tasks that require long durations of attention, which is critical for this research program.

Therefore, to understand the neuronal basis of human visual perception, attention and visually guided behavior, primates must be used. However, the availability of naïve primates for purchase has been dramatically impacted by diversions to COVID research and the cessation of animal export by China. In addition, a May presentation by Dr. Arnegard of the NIH's ORIP at the Simian Collective Meeting described a 4-5 year lag in the NPRC breeding program. The WUSTL veterinary staff and faculty have been actively, but unsuccessfully, pursuing multiple potential purchase options. Allowing [redacted] to continue to participate in [redacted] research program will prevent prolonged gaps in establishing a successful and productive lab.

[redacted] – [redacted] participated in six (6) procedures (head post implantation; recording chamber implantation; artificial dura implantation; viral injection; pial peel; chamber removal) at the [redacted] prior to arrival at WUSTL. Procedures were limited to the left primary visual cortex. At WUSTL, [redacted] will continue along the originally planned experimental pathway and participate in procedures identical to the previous research plan at the [redacted]. A recording chamber would be positioned over the right extrastriate visual cortex. [redacted] would undergo an estimated three survival surgery procedures at WUSTL (right hemisphere chamber implantation, artificial dura implantation, chamber removal). If additional surgeries are needed to repair or remove implants, the veterinary staff will be consulted. [redacted] would continue to participate in research as described in the approved WUSTL protocol. The timeframe would be no more than 15 years of service or the humane endpoint, whichever is earlier. Total years of service would be calculated as the sum of research participation at [redacted] and WUSTL.

Justification: The six major survival procedures performed at the [redacted] successfully developed an optogenetics approach with a transparent artificial dura to depolarize neurons in the visual cortex while an animal is doing a task. At WUSTL, [redacted] will expand on this initial work using the same optogenetic approach in a recording chamber positioned over the right extrastriate visual cortex. While the same lack of available nonhuman primates applies to the proposed exemption request for [redacted], the continued application of this optogenetic technique in the same animal will enhance the scientific validity of the data collected. In addition, [redacted] previous experience with similar behavioral tasks will allow the current project to proceed with minimal training periods.

If the exemption request is approved, the IACUC will submit annual reports to me or the current Institutional Official at the time of the report. The report will include an assessment of the animals and an evaluation of the procedures and methods used. Any approved exemptions will be included on the Annual Report (Form 7023) submitted to APHIS.

Thank you for your consideration. Please do not hesitate to contact me if you have any questions or would like additional information.

Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8106-0016-10, St. Louis, Missouri 63110-1093
Phone: (314) 747-0515, lowe@wustl.edu

Sincerely,

(b) (6), (b) (7)(C)

Dr. Mark Lowe, MD, PhD
Institutional Official
Interim Vice Chancellor for Research
Interim Associate Dean for Research, School of Medicine
Harvey R. Colten Professor of Pediatric Science

Washington University in St. Louis
660 South Euclid Avenue
Campus Box 8106
Saint Louis, MO 63110

Washington University in St. Louis
Institutional Animal Care and Use Committee

IACUC Protocol Approval
Animal Welfare Assurance #D16-00245
September 01, 2022

PI: (b) (6), (b) (7)(C)
CO-PI:
From: (b) (6), (b) (7)(C)

Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

Protocol No. 22-0184
Species: Non-Human Primate (various)

Funding

Agency/Title: National Eye Institute/NIH/DHHS/NEI
Border ownership and grouping in primate visual cortex
Agency/Title: Neuroscience (003021)
Startup funds

The Institutional Animal Care and Use Committee of Washington University at St. Louis has approved this protocol for the use of animals in conjunction with the research project(s) named above.

Protocol Approval Date: September 01, 2022
Protocol Expiration Date: August 31, 2025

As the PI, it is your responsibility to ensure the following:

-- All personnel must follow the approved protocol and limit activities to only those listed on the protocol.

-- Modifications to the approved protocol must be approved by the IACUC before they are implemented.

-- Adverse events, unexpected problems, and protocol deviations must be reported to the IACUC. Please contact the IACUC office at iacuc@wustl.edu or 314-362-3229 for assistance with reporting.

-- All personnel must be sufficiently trained. Training classes in animal handling, procedures, and aseptic technique are available through the DCM training office. Contact DCM for scheduling assistance.

Failure to comply with these provisions may result in suspension of the protocol. If you have any questions on how to

implement these requirements, please contact the IACUC office. We appreciate your efforts to maintain the highest standards of animal welfare.

Campus Box 1054, One Brookings Drive St. Louis, Missouri 63130,
(314) 362-3229, Fax: (314) 747-6695, https://wustl.keyusa.net

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Protocol Title:	Mechanisms of visual perception, attention and visually guided behavior in the primate brain
Protocol Status:	APPROVED
Date Submitted:	06/30/2022
Approval Period:	09/01/2022-08/31/2025
Important Note:	This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol. Questions that appear to not have been answered may not have been required for this submission. Please see the system application for more details.

* * * Personnel Information * * *

Personnel Information

Personnel Types include PI, Co-PI, Protocol Contact, Staff Personnel with Edit/View Access, and Other Personnel with View Only. Access and permission details for each personnel type are listed below.

Click "Add" to assign personnel in the appropriate sections. Use the binoculars icon in the pop-up window to search the Directory by first or last name. Leave a section blank if you do not have personnel in the category described.

If an individual does not appear in the search results, contact the IACUC Office 314-362-3229

Individuals who are not listed on this page cannot handle animals housed on this protocol unless a specific collaboration is approved on the General Questions page.

Principal Investigator

The Principal Investigator can VIEW, EDIT, and SUBMIT.

Principal Investigator

Name*		Title
[redacted]		Security Access Only
Email*		Office Phone
[redacted]@wustl.edu		
Lab Phone		Cell Phone
		[redacted]
Department*		Emergency Phone
Neuroscience Core		[redacted]
Degree		Personnel Type
MD PhD		Security_Access_Only

Does this Protocol involve the use of Controlled Substances in live animals?*	Y
Is the PI working with live vertebrate animals?*	Y
If "Yes" answer the following:	
What Species is the PI handling on this protocol?	Non-human primate (macaque)
Is the PI performing Surgery?	Y
Is the PI administering anesthesia to animal?	Y
Provide a summary statement of the PI's training. Include: *	

Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

•	years of experience for each species
•	a brief list of procedures the PI will perform on this protocol
•	name of trainer if additional training is needed

Examples are available in the Help text

Note: List procedure names or "all procedures". Details are collected in other sections of the protocol.

PI will perform all procedures. PI has over 12y experience with surgical and physiological procedures in various mammalian species including Macaca monkeys, and 6y of full-time experience with NHP of genus Macaca, both with behaving animals as with animals under anesthesia. PI has also completed clinical residency in neurology in human patients, including a rotation in neurosurgery.

Prior to approval, all individuals listed on an IACUC Protocol are required to complete the required core training course title(s). See the "https://research.wustl.edu/topics/animal-care-use/" target="_blank" website link policies for more info.

Training Details

CourseID	Course	CourseCompletionDate
182	DCM - Introduction to Animal Care and Use	17-JUL-2022
190	DCM - Non-Human Primates	07-JUN-2022
178	DCM - Rodent Barrier Orientation	07-JUN-2022
00003082	OHS - Medical Surveillance Questionnaire for Personnel with Animal Contact	17-JUL-2022

Co-Principal Investigator

The Co-Principal Investigator(s) can VIEW and EDIT the protocol.

Primary Protocol Contact

The Protocol Contact can VIEW and EDIT the protocol. They are copied on all protocol email communications. This individual may act on behalf of the PI.

Staff Personnel - Read & Write Access

Staff Personnel can view and edit protocol, but are not copied on email notifications.

Other Personnel - Read Only Access

Other Personnel can only view the protocol.

See research.wustl.edu/lamps for system help and training resources.

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* * * Species * * *

Species

This is a mandatory section. You must add at least one species to continue.

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To delete a species, you must first delete any procedures associated with that species on the Procedures Page.

Species to be Used

Species Common Name	Pain Classes	Total
Non-Human Primate (various)	D	8

Species to be Used

1. **Species Common Name*** Non-Human Primate (various)
2. **Scientific Name** Various
3. **Animal Sex*** Both
4. **Housing Location** Washington University in St. Louis

Justification for Choice of Species

5. **Why are the selected species the most appropriate for these studies?***

The experimental animals for this research program are monkeys of genus Macaca. The possible species used are Macaca mulatta (rhesus), Macaca fascicularis (cyno), Macaca nemestrina (southern pig-tailed macaque). The experiments carried out in the course of this program are designed to elucidate the neural mechanisms and events underlying visual perception, attention and visually guided behavior. This program requires a member of the genus Macaca because (1) all of the prior work upon which the program is based used the macaque; (2) the phylogenetic proximity of Old World monkeys to humans and close similarities of their brains to the human brain, especially for the visual system, make the results of our experiments relevant to an understanding of human vision and cognition, and to the development of clinical procedures for treatment of disorders of these systems. For example, humans and nonhuman primates differ from rodents in how they explore the visual environment. The primate oculomotor system serves to move the eyes to align the high-resolution fovea with objects of interest in a scene. The presence of the fovea thus changes in a fundamental way how primates use their eyes to acquire information about the world. (3) The macaque is capable of performing complex tasks, including tasks that require long durations of attention, which is critical for this research program. In conclusion, to understand the neuronal basis of human visual perception, attention and visually guided behavior, primates must be used. There has been more background work done on the mulatta species than any other. However, differences between macaque species that may be used (M. mulatta, M. nemestrina and M. fascicularis) are relatively minor, so depending on cost and availability we may substitute one of the latter two species.

6. **Animal Numbers***

USDA Pain Category - Choose all that will apply. For each applicable pain category enter the total number of animals to be used during the 3 year lifetime of the protocol. If animals will be used in more than one category, enter the number in the higher category. Scientific justification for Class E procedures will be collected in a different section.

EXAMPLE: If 150 mice will be used in Category C for ear punching and 50 of those mice will then be used in Category D for laparotomy, list 100 mice in Category C and 50 mice in Category D.

Breeding - All animals bred in-house must be counted and included in your Animal Quantities, including any surplus or unsuitable animals that will be born but not used for experiments.

Field Study - If this is a field study, enter the number 1 in the appropriate category to indicate animals will not be ordered. The total number of field study animals participating in your study will be described in the justifications for numbers section.

	Pain Category C	
X	Pain Category D	8
	Pain Category E	

7. **Total Number of animals requested for this species (3 year total)*** 8
8. **Animal Identification (select all that apply)** Cage Card Only,Tattoo

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Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

"https://research.wustl.edu/animal-identification-guideline/" target="_blank" Animal Identification Policy

Description of Phenotypes (ADVERSE PHENOTYPES)

9. Will any naive animal strain develop a phenotype with adverse clinical consequences impacting the health of the animal [Example: paralysis, malocclusion, skin lesions, tumor development]?* N

For only those strains expressing a phenotype that would negatively affect the health of the animal, please address the following questions. Identify each response by strain or genotype.

a. Describe the phenotype and any pain or distress associated with its manifestation. Specify when the expression of the phenotype is expected and the age of the animals you will be using in the study.

b. Describe the procedure(s) proposed to avoid or alleviate pain. Responses may include the following: palliative care measures or treatments, modifications to husbandry procedures, monitoring schedules, criteria for assessment (body condition scoring, tumor scoring, etc.), humane endpoints.

See research.wustl.edu/lamps for system help and training resources.

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* * * Lay Summary, Sequence & Timing * * *

Lay Summary, Sequence & Timing

Client Protocol ID (for office use only)

PROTOCOL TITLE

Protocol Title *

Mechanisms of visual perception, attention and visually guided behavior in the primate brain

LAY SUMMARY

Study Objectives

1. Lay Summary of Project Goals and Significance - Use language understandable to a layperson as you answer questions in this section. Avoid overly technical terms and define abbreviations. Lay summary examples are available in the help text for this page.

   a. Provide a brief synopsis of each research project covered by this protocol. The summary should include the overall objective(s), and the potential benefits to human/basic knowledge, and how animals will be used to achieve the scientific objective.*

The goal of our research is to understand how the brain enables us to perceive, understand, attend to and engage with the objects in the world around us. This is not a trivial task for the brain. Our eyes capture light that is reflected by the external environment, but these light particles (photons) do neither unambiguously indicate whether they are reflect from background or an object, nor what type of object they are reflected from. For example, the color spectrum of reflected light depends on the properties of the surface that reflects it, but also on the spectrum of the incident light that falls on this surface, thus any change in the spectrum of reflected light captured by our eyes could indicate a change in either, or a combination of the two. Our brain must thus perform complicated computations to infer the most likely organization of the objects around us, and their identity. This is critical to be able to interact with the external world.

We need to understand these brain mechanisms in order to understand and develop treatments for brain disorders in which perception and attention fail. Schizophrenia, Autism and Alzheimer's Disease are examples of brain disorders in

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which these processes are severely impaired. By understanding the brain mechanisms that fail in these disorders, we will provide a basis for developing improved diagnostic and therapeutic techniques for dealing with the neurological problems of human patients.

Single and multiple unit recording techniques will be used to study the underlying neural computations. Such procedures will result in the development of precise models that can be tested using computer simulations. Further insights can come from observing the effects of temporary inactivation or temporary over-activation of particular regions of the brain and these can be especially relevant to our understanding of human neurological disease. In addition, the anatomical characterization of the neural structures under study is essential for a clear formulation of brain function. This will be studied using advanced viral targeting and optical approaches (including widefield imaging and multiphoton imaging), which allow to link function to anatomical organization with high resolution. On occasion, this will be combined with traditional anatomical methods, to get cell specific information about circuits, allowing for more precise modeling of neuronal functions, particularly relevant to human diseases.

b. **Justification for using animals in research**

Explain why vertebrate animals are required to accomplish the project goal(s). Explain why those goals could not be achieved using invertebrates, in vitro experiments, or computer models.*

Our research requires both complex behaviors and intact biological systems to answer the scientific questions asked. Invertebrates and lower species do not exhibit the behavioral or biological complexity necessary. Computer models do not replicate the systems studied. At present, no other experimental techniques provide a detailed understanding of how the activity of individual neurons leads to complex perception and behaviors. In particular, non–invasive methods such as EEG and fMRI only provide measures of global activity, and lack the spatial and/or temporal resolution necessary to measure the spiking activity of individual neurons.

Please note: You may copy and paste plain text from a document into the text box, but the formatting will not transfer.

Recommendation: When copying text from a Word or PDF file, it is recommended to first paste into Notepad, and then copy and paste into the eProtocol text box.

SEQUENCE & TIMING

2. Flow Chart - For each experiment involving animals, provide a flow chart with a clear and concise sequential description of the procedures involving live vertebrate animals. Describe the chronological sequence and timing of all the manipulations. Include numbers used for each group of experiments, drugs and substances administered, the time between procedures, and experimental endpoints. Do not describe how the procedures are performed as this information is collected in another section. Reviewers must be able to understand what each animal will experience while housed on this protocol. Please separate paragraphs with a blank line.*

If you have tables or diagrams, please use the Add feature to attach the document in the Attachment section.

Animals will be purchased and allowed to acclimate to DCM housing prior to use. Acclimation to permanent housing location, their neighbors, lab personnel, and schedules may take several days to several weeks.

Experiments are designed as follows:
1. Baseline neuroimaging procedures are typically conducted on naive animals.
2. Begin basic handling and restraint training -- generally several weeks to several months.
3. Implant head post. Recovery period is estimated at 1 to 6 weeks.
4. After recovery from surgery, animals will begin water restriction for task performance training.
5. Desensitization to head-stabilization and task performance training -- 1 to 12 months depending on task complexity.
6. Before physiological experiments begin, we will implant one or more recording chambers. A craniotomy is performed, keeping the dura intact, and the chamber is inserted and cemented in place. Post-surgical recovery is typically 3-14 days. To prevent dural thickening prior to installing the artificial dura (7), the chamber will be opened 2-3 times per week to apply 5-FU, and thoroughly rinsed with sterile saline.
7. To enable the insertion of thin multielectrode silicon probes, or deliver light for optogenetics or for optical imaging, we will often perform a durotomy to replace the native dura with a transparent artificial dura. This procedure is typically done in a separate surgical session, but it may be combined with the craniotomy if time allows. Post-surgical recovery is typically 3-14 days. As soon as the

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with the craniotomy if time allows. Post-surgical recovery is typically 3-14 days. As soon as the durotomy has been performed and the artificial dura installed, the chamber will be opened and rinsed with sterile saline at least every five days. In chambers without an artificial dura, cleaning will occur at least weekly.
8. For some experiments (e.g. to express opsins or calcium indicators, resp. for optogenetics or for optical imaging of calcium signals), we will perform viral injections in the brain. This is often done in a separate procedure, but may be combined with durotomy if time allows. It may also precede chamber implantation, so that protein expression is present as soon as the chamber is implanted. Recovery is typically 3-14 days.
9. Physiological experiments begin. This may include behavioral and/or electrophysiological recording with or without electrical stimulation or optogenetic stimulation, and/or optical imaging.
10. Additional anatomical imaging may be done after experiments have begun to guide subsequent recordings and verify electrode position. (e.g., imaging with in situ electrode).
11. For experiments with artificial dura chambers in which good optical access is necessary (e.g. optogenetics, optical imaging, precise placement of electrode probes), a pial peel procedure may be necessary over time. Tissue often builds up in between the artificial dura and the pial surface over time, which requires removal in order to collect data in such cases.
12. If recording from a chamber is no longer necessary, the chamber is often removed if possible, to contribute to the animal's comfort by reducing the number of chamber cleaning sessions. The animal may continue in behavioral experiments after the chamber is removed. If instead data collection in a chamber is no longer necessary, but the animal does not need to participate in more behavioral experiments nor in recording sessions from another chamber, we would not remove the chamber, but proceed to step 14, because the time until euthanasia in those cases would be short.
13. Monkeys may participate in multiple interrelated behavioral tasks as 4-12 continue.
14. If histological data is needed, animals may have viral injections in separate sites for histology and/or electrolytic lesions, followed by euthanasia and transcardial perfusion.
Cycle 4-12 may be repeated (described in detail below). Perfusion at the termination of the experiments, required for anatomical localization of recording sites and tract tracing, is performed after anesthetic overdose.

Humane endpoints:
We will use most animals for 10-15 years (2 to 3 NIH R01 grant cycles). Macaques in this type of study can provide valuable scientific data for years. Previously, animal endpoints were usually defined by a need for timely tissue examination to confirm recording or injection sites, or as a necessary step in tract-tracing experiments. Currently, the artificial dura approach allows precise placing of electrodes or injections on the cortical surface, which, in combination with data from anatomical scans, often allows to postpone or replace a post-mortem tissue exam. With these technical advancements, endpoints are often defined by adverse clinical symptoms and signs. Animals that exhibit symptoms of severe infections, persistent infection unresponsive to treatment, signs of stroke or significant neuronal impairment, or adverse clinical symptoms preventing behavioral or task performance will be referred to the veterinary staff for evaluation and euthanasia, if necessary.

Housing prior to use:
Procurement times vary from 4 months to one year (due to backlogs on animal availability, shipping schedules and quarantine time). Because animals are almost always ordered in pairs so that they can be pair-housed, animals must be ordered well in advance of when they will be used, and as a result may be kept for one to two years before they begin training. Animals may remain naïve and not used for experiments until they reach sexual maturity. This is done in our (and all other NHP labs that we are aware of that use complex tasks) because younger monkeys are difficult or impossible to train for complex tasks, do not display accurate oculomotor behavior (saccades) in tasks, and are not ideal for surgery for technical reasons. Furthermore, NHP vendors typically do not have older monkeys for purchase. For such animals that are held because they are too young for surgeries and task training, additional enrichment will be provided to address the animal welfare concerns associated with confinement and inactivity. This includes starting pole and collar training with regular positive human interactions, and additional enrichment (rotating schedule of toys, food items that encourage foraging and task-oriented feeding).

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Participation in multiple experiments:
Experiments and data collection may take several years. Training on a complex task may take a year or longer. This includes everything from pole-and-collar acclimation to reaching a steady state behavior, which is often required before physiological study can begin. Next, recording physiological data can take two or even three years. Finally, peer reviewers and journals may require that additional data or controls be collected, which generally must be done in the same animal. This requires that the animal be maintained while the study is being written up and through the review process.
Using an individual animal for more than one experimental procedure is often necessary. (Here we distinguish experimental procedures from surgical procedures. The former include but are not limited to tracking behavior in a particular task, recording physiology in a particular task and from a particular brain region, electrically or optogenetically intervening in one region and either tracking behavior or recording electrophysiology in another, etc.) Most experimental aims entail multiple individual procedures. In many cases, these procedures must be performed in the same animal to be scientifically valid. In the field of systems neuroscience, individual subject differences are key. Thus, we cannot record from region A in one monkey and region B in a second and put those data together in order to build models of the circuitry connecting areas A and B –the recordings must be from the same animal. Hence, in each individual, neuronal recording from different brain regions (sometimes sequentially and sometimes simultaneously) is vital to understanding how neuronal circuits result in complex perception within an individual.
Each additional data set from any given animal becomes exponentially more valuable. The more information we have about how that particular animal performs and how neurons throughout the brain of that animal are activated during those tasks, the better we can model how those neurons work together to generate the behaviors of interest. Often, we cannot plan in advance exactly which brain areas we will target, or even which tasks we will employ. This depends on the results from each study. As a result, we often add new chambers to an animal (along with craniotomies) to access an area that we originally did not know would be crucial for a particular experimental sequence. Unused craniotomies will close naturally over time, causing no discomfort for the animals in the process.
There are additional advantages of using a single animal for multiple procedures and long time spans. First, NIH review committees often assume that animals trained and instrumented in a previous grant cycle will be available for use in the next grant cycle. Second, using animals for multiple experiments helps us meet the goal of animal number reduction indicated by the Guide for the Care and Use of Laboratory Animals.

Number of neuronal recordings.
Electrophysiological recording
Neuronal recordings in neurophysiology labs are done approximately five times a week. However, the total number of recordings and associated penetrations depend on training status of the animal, objective, and online parameters of the experiment that cannot be predetermined (for example the number of neurons on electrode contacts, the functional nature of those neurons, or the location of the recordings). The current experimental practice across all NHP labs that the PI has trained in and is aware of in the field (and at WUSTL) have shown that repeat neurophysiological recordings with micro-electrodes is safe and well tolerated and does most often not lead to changes in behavior of the animal (as expected by the fact that small changes, if any, in the brain are known to produce no behavioral or sensation-like effects in humans during neurosurgery which include larger electrodes and more marked histological changes). Most importantly, experiments in any one given brain area or animal are self-limiting. Because we study "normal" healthy brain function, we consider on a daily basis whether there are any changes in neuronal responses or behavior. Our experience is that changes in behavior are exceedingly rare. The number of neurons encountered per penetration may drop over time with particularly intensive recording (e.g., after several hundreds of penetrations into a single small area over a span of one or more years). When we observe such an effect, we stop recording from that area, long before there are any behavioral or clinical effects.

Optical imaging
As for electrophysiological recordings, optical imaging sessions are done approximately five times per

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week, but the total number of sessions depend on training status of the animal, objective, and online parameters of the experiment that cannot be predetermined. In contrast to electrophysiology, there is no penetration of brain tissue during imaging sessions.

Note: You may copy and paste plain text from a document into the text boxes (Formatting will not transfer into the text box).

Recommendation: When copying text from a Word or PDF file, it is recommended to first paste into Notepad, and then copy and paste into the eProtocol text box.

If you have tables or diagrams that will assist with the understanding of the experimental animal groupings, please use the Add feature to attach the document in the Attachments section. Reference the name of the attachment in the text of the description where appropriate.

3. **Indicate how GROUP sizes (number of animals per group) were determined and explain below. This answers the question "How did you determine that the group size should be n=X?" Check all that apply.**

Number of animals based on quantity of harvested cells or amount of tissue required.

Number of animals determined statistically. Describe the statistical analysis.

Pilot study - group variances unknown at present

X Other

We make every effort to use the fewest number of animals possible. In practice, this means that we typically use 2 animals in each study. The reason for this number is as follows. The most fundamental requirement to make any scientific statement is that the evidence supporting that statement must be reproducible. In other words, if the experiment is repeated, the results must be the same. If this criterion is not met, the "results" do not qualify as science. The minimum number of animals necessary to verify that our results are reproducible is 2. Thus, we require that the same behavioral/physiological results are obtained in the 2 animals. To avoid introducing differences within a single experiment that may impact statistical power, these two animals will typically be of the same Macaca species. This criterion also guides the policy in peer-review scientific journals. High-profile journals in neuroscience require at least 2 animals for publication. Occasionally, the first 2 animals provide different results. If this happens, we will test one or two additional animals in the same study. This protocol will cover multiple tasks, closely related to each other. Unless there is an urgent need for histological data, each animal may participate in multiple tasks. Because subsequent tasks are closely related, the behavioral tasks have many aspects in common. Thus after the initial training, animals can learn new tasks often much faster. This greatly reduces the total number of animals needed over a period of years.

4. **Based on the group sizes determined above, describe how you arrived at the total number of animals used on this protocol.**

Use the Add feature to attach tables or diagrams in the Attachments section. Remember to reference the names of any table or diagram attachments in the narrative below. *

Because great effort goes into training each animal, we are highly motivated to minimize the number of animals used. Typically, 4 different tasks will be underway simultaneously in the laboratory, each requiring 2 animals. The total census of animals of genus Macaca for this protocol is 8, but the balance between different Macaca species (mulatta, fascicularis, nemestrina) may differ depending on availability.

If you have tables or diagrams, please use the Add feature to attach the document in the Attachments section.

*** General Questions ***

Protocol Resubmission

1. **Are you renewing a protocol for another three (3) years?*** N

   a. **If yes, please enter the protocol number you are renewing.**

   b. **Provide a brief summary of accomplishments from the past three (3) years (completed experiments, discoveries, abstracts, presentations, publications) and a justification for continuing this project.**

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Videos, Photographs, and Electronic Media

2. Will videos, photographs, or electronic media of experimental animals be recorded?* Y

   a. If yes, please describe use and how the materials will be shared. Recorded materials must be stored in a secure location (e.g. locked desk or cabinet in a locked room).

   Storing Sensitive Material - Use & Dissemination of videos, photos, or electronic media Policy

   Pictures and video recordings of individual monkeys will not be made. Local high-resolution images of brain tissue will occasionally be made such as during optical imaging sessions and/or to illustrate the regions targeted with electrodes or viral injections, which often need to be included in peer-reviewed publications. In addition, images may be made if particular veterinary issues need to be shown to the veterinary staff. All these images will be stored in a secure location.

Custom Antibody Development and Production

3. Will you contract with a separate company or institution to develop a custom antibody? [Example: Sending an antigen to a company for injection into rabbits with blood collection - answer "Yes". If you are purchasing an antibody from a catalogue - answer "No"]* N

   a. If yes, please provide the name of the company and the OLAW Assurance number.

Collaborations (includes WUSTL Core Facilities, WUSTL Investigators, and Other Institutions)

4. Does this protocol include collaboration with other WUSTL PIs, WUSTL Core Research Facilities, or with other institutions?* N

   Policy on Core Research Collaboration

Breeding

5. Will animals be bred in-house?* N

   All animals bred in-house must be counted and included in your Animal Quantities on the "Species" section, including any surplus or unsuitable animals that will not be used for experiments.

   Rodent Cage Space & Weaning Policy

Field Studies

6. Is this a Field or Wildlife Animal Study Protocol?* N

   Your protocol will be forwarded to the Occupational Health and Safety office because the hazards and risks of fieldwork are different from those in a laboratory setting. They will contact you and your personnel directly to discuss the risks associated with working with wildlife, such as animal bites, zoonotic disease transmission and exposure to allergens. Additional PPE or vaccines may be required based on species or location.

Controlled Substances - Proposed Total Volume Used

7. Will your research require the use of controlled substances? If yes, list the proposed total volume used during the 3-years of protocol approval for each controlled substance or drug* Y

   Buprenorphine SR - 55 mls (3 mg/mL)

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Buprenorphine HCl – 28 mls (0.3 mg/ml) Diazepam (Valium) - 492 mls (5 mg/mL) Ketamine - 123 mls (100 mg/mL) Pentobarbital - 180 mls (50 mg/mL)

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* * * Procedures * * *

Procedures

1.	**Procedure Type:***	Training and Task Performance
2.	**Brief Description:***	NHP Handling and Daily Routine
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	C

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

Each monkey lives in a home cage without restraint, but wears a neck collar, to which we can attach a pole for handling the animal. All members of the lab are trained by the PI or experienced senior lab members to handle monkeys in this way safely. The monkey is guided from the cage by the pole to enter a primate chair. We will use only a rigid pole to handle conscious macaques and will follow the IACUC policy, "Acclimation of Nonhuman Primates to Experimental Restraint".
The animal is weighed (at least once a week during periods of fluid restriction) and transported to the lab for a training or experimental recording session, which typically lasts 4–8 hours (including the time required for chamber maintenance before and after recording sessions that involve electrophysiological recordings, optogenetics or optical imaging). The primate chair has multiple degrees of adjustment and allows the animal to sit in a comfortable posture.
Urine and feces are collected in a wood–chip filled pan below the chair. The monkey is able to move its limbs and trunk freely within the primate chair, and is returned to its home cage at the end of each session. During the session, the animal's head is held firmly in place by means of a head post that is implanted on the skull and that attaches to a head bar mounted on the chair.
Usually within a two–week period of initial training, the animal becomes comfortable with the daily routine of chairing, weighing and head–fixing. Once acclimated, the monkeys enter the chairs readily under their own power.

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

It is possible that some animals do not acclimate to restraint.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

N/A

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

Animals that do not acclimate to restraint will be removed from study procedures that include the restraint, transferred to another investigator, or re-introduced to the restraint after a sufficient rest period discussed with the veterinary staff.

5. **What is the duration of the procedure, from anesthesia to wake up?**

N/A

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Procedures

1.	**Procedure Type:***	Training and Task Performance
2.	**Brief Description:***	Routine Maintenance of Recording Chamber With Artificial Dura
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	C

* * * Procedure Description * * *

Procedure Description

1. Detailed Procedure Description

The inside of the recording chamber must be kept clean and aseptic to avoid infectious agents that might compromise the health of the tissue and limit visibility of the brain through the artificial dura. To this end, we will clean both wound margins and open/clean the chamber at least every 5 days in animals that are not also being used in physiological procedures, and during each recording session for those animals who are being used in physiological procedures (at least every 5 days). Experience in the (b) (4) laboratory at the (b) (4), where this chamber design is routinely used and where the PI was trained, as well as in other labs, shows that cleaning of the inside of the implanted chamber can be done at these intervals without adverse effects.
The animal is brought to the lab in the primate chair and its head is held stationary using the head post. The outer surface of the chamber and the surrounding cement and skin are cleaned thoroughly with in sequence Nolvasan, betadine and 70% alcohol. If an animal's wound margin around the chamber is sensitive, we will apply topical Lidocaine 2.5%/Prilocaine 2.5% to the skin to numb it during cleaning. Veterinary services will be notified if the sensitivity appears to be due to an allergic reaction; treatment plan will be discussed with the veterinarian(s). After cleaning the outer surface of the chamber and surrounding cement and skin, the cap of the recording chamber is removed and placed into hydrogen peroxide. We will ensure that there is a sterile field around the chamber, retracting any tissue that might otherwise intrude into the field such as the outer ear. An autoclaved chamber cleaning kit is then opened aseptically, and the investigator wears sterile surgical gloves along with a face mask, shield and hair cover. The chamber is thoroughly irrigated with sterile saline. The solution is removed from the chamber using a sterile pipette attached to a sterile vacuum line, with a trap, or using sterile gauze and sterile cotton applicators. We use a sterile cotton applicator and sterile forceps to remove any tissue that forms between the chamber and the artificial dura. Over time, it is possible that the brim of the artificial dura, which sits underneath the edge of the native dura, gets pushed out of the durotomy due to build-up of granulation tissue. If this happens, we replace the artificial dura with a new, sterile, brimless artificial dura. On recording days, after irrigation, the chamber is prepared for recording (by inserting a sterile stabilizer insert for electrophysiological recordings, optogenetics or imaging, and/or a sterile imaging platform to the chamber for optical imaging). At the end of the recording session, the chamber is thoroughly irrigated with sterile saline again as described above. A small piece of sterile gauze soaked with topical antibiotic is left in the chamber (on top of the artificial dura), and a sterile silicone plug, to ensure the stability of the artificial dura between cleanings. The chamber is then closed again with an autoclaved cap including a sterile silicone gasket to seal the chamber. A small amount of melted bone wax is typically applied on the outside between chamber cap and chamber as an additional seal.

The use of amikacin (or gentamicin) is restricted to the very specific situation of a recording chamber in which the native dura has been replaced with a transparent membrane (artificial dura). The artificial dura remains in place by use of a brim that sits underneath the edge of the remaining native dura underneath the chamber edge. This approach is critical for certain experiments in our lab (e.g. optical recording or perturbation of tissue at the cortical surface, precise insertion of multielectrode probes while avoiding blood vessels), as well as those in several other NHP labs (see below).
In this case, an important barrier against infection, the dura mater, has been removed. In addition, without the dura it is not safe to use antiseptics during chamber cleaning, as in standard recording chambers. Therefore, after copious rinsing with sterile saline under aseptic conditions, we leave a small amount of amikacin (or gentamicin; ~0.2 mL) on a piece of sterile gauze on top of the artificial dura before closing the chamber. This method of cleaning is standard in the field: it is used in all the laboratories that use artificial dura recording chambers in macaques as far as we know. These laboratories include the (b) (4) (b) (4) (b) (4), (b) (4), (b) (4), (b) (4), (b) (4), (b) (4). It has been described in several peer-reviewed publications from different labs (e.g. Ruiz et al., J Neurophys 2002; Roe, Appl Opt 2007; Yazdan-Shahmorad et al., Neuron 2016; Chernov et al., J Neurosci Methods 2016). Together with the meticulous aseptic cleaning as described, this has led to a virtually perfect track record during the 10 years of experience with artificial dura chambers in the (b) (4) (b) (6), (b) (7)(C). Also experience in the other labs mentioned above shows that this approach is safe. In addition, the experience in the field has been that if amikacin is not applied, it leads to a substantial increase in risk of infection (b) (6), (b) (7)(C) personal communication). Therefore the available evidence indicates that this is the best approach to maintain these chambers.

Chamber cleaning prior to durotomy
There is typically a period of 2 weeks between chamber implantation and durotomy/artificial dura implantation. During this period, during the cleaning procedure, 5-fluorouracil (5-FU) in saline (250 mg/10 mL) is typically applied on top of the dura to slow down the build-up of granulation tissue, during 5 minutes, typically 2-5x per week. 5-FU is an anti-mitogenic agent that blocks nucleic acid

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build-up of granulation tissue, during 5 minutes, typically 2-5x per week. 5-FU is an anti-mitogenic agent that blocks nucleic acid synthesis thereby retarding tissue growth. The use of 5-FU for this purpose is described in detail in a methods paper that was published in the Journal of Neurophysiology (R.L. Spinks et al, Journal of Neurophysiology, volume 90, pages 1324-1332, 2003). Extensive testing by the authors of this paper revealed that while 5-FU is very effective at retarding the growth of granulation tissue over the craniotomy, it has no measurable effect on the neuronal tissue underlying the dura. The authors have done histology on 3 monkeys after regular application of 5-FU, and no damage to the underlying tissue was evident in any monkey. They regularly record the responses of neurons in cortex under craniotomies that are regularly treated with 5-FU, and find robust neuronal activity as soon as the electrode enters cortex, indicating that neurons remain healthy and active after repeated 5-FU treatments. After 5-FU application, the chamber is thoroughly irrigated with sterile saline. Experience in the [redacted] shows that 5-FU treatment aids the ensuing durotomy (smaller volume of tissue needs to be removed, which results in a shorter surgery duration), without adverse effects.

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

The major potential complications involve infections along wound margins or under the implant. Work in the [redacted] at the [redacted] – where these type of chambers are installed and maintained routinely, and where the PI was trained - and in several other NHP laboratories, has shown that the artificial dura system is very effective at protecting the brain against infection, if properly maintained, as described above. Second, we will carefully monitor the health of the chamber, and if infection (confirmed by taking a culture inside the chamber) should occur that we, working with DCM veterinary staff, cannot control, the implant will be removed. In rare cases, the head post may loosen or detach.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Recording chambers are cleaned daily when the animal is being recorded from, or at minimum every 5 days if the animal is not being recorded from. We clean the implant flushing it liberally as described above. In addition to the routine inspection and cleaning of skin margins, we apply topical antibiotic ointment if there are signs of infection, and we consult the DCM staff about appropriate topical and/or systemic treatments to be used.

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

As described in the FlowChart, Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

This is performed in awake animals and typically takes up to 60 minutes, including the time to transport the animal. This procedure is typically performed in awake animals who are restrained in a primate chair, as described above, and head fixed for the safety of the animal and researcher. In some cases, the chamber will be cleaned while the animal is anesthetized (e.g. on days when a chamber is due for cleaning and the animal happens to be in surgery).

Procedures

1.	**Procedure Type:***	Training and Task Performance
2.	**Brief Description:***	Routine Maintenance of Recording Chamber Without Artificial Dura
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	C

* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

We will clean wound margins and open/clean the chamber at least weekly in animals that are not also being used in physiological procedures and during each recording session for those animals who are being used in physiological procedures (at least weekly). The animal is brought to the lab in the primate chair and its head is held stationary using the head post. The outer surface of the chamber and the surrounding cement and skin are cleaned thoroughly with in sequence Nolvasan, betadine and then 70% alcohol. If an animal's wound margin around the chamber is sensitive, we will apply topical Lidocaine 2.5%/Prilocaine 2.5% to the skin to numb it during cleaning. Veterinary services will be notified if the sensitivity appears to be due to an allergic reaction; treatment plan will be discussed with the veterinarian(s). After cleaning the outer surface of the chamber and surrounding cement and skin, the cap of the recording chamber is removed and placed into hydrogen peroxide. We will ensure that there is a sterile field around the chamber,

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recording chamber is removed and placed into hydrogen peroxide. We will ensure that there is a sterile field around the chamber, retracting any tissue that might otherwise intrude into the field such as the outer ear. An autoclaved chamber cleaning kit is then opened aseptically, and the investigator wears sterile surgical gloves along with a face mask, shield and hair cover. The chamber is thoroughly irrigated with sterile saline, dilute Nolvasan (0.05%) and/or Dakin's solution (1% bleach). The solution is removed from the chamber using a sterile pipette attached to a sterile vacuum line, with a trap, or using sterile gauze and sterile cotton applicators. We may apply 5-FU, an antimitotic agent, within the chamber to retard the formation of granulation tissue, followed by liberal flushing with sterile saline (see Routine Cleaning of Recording Chamber With Artificial Dura, section Chamber cleaning prior to durotomy). We may also use sterile forceps and cotton tip applicators to remove soft granulation tissue. The chamber is dried using sterile cotton tip applicators. The chamber is then closed with an autoclaved cap including a sterile silicone gasket to seal the chamber. A small amount of melted bone wax is typically applied on the outside between chamber cap and chamber as an additional seal.

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

The major potential complications involve infections along wound margins or under the head implant. In rare cases, the head implant may loosen or detach.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Recording chambers are cleaned daily when the animal is being recorded from, or at minimum weekly if the animal is not being recorded from. We clean the implant flushing it liberally as described above. In addition to the routine inspection and cleaning of skin margins, we apply topical antibiotic ointment if there are signs of infection, and we consult the DCM staff about appropriate topical and/or systemic treatments to be used.

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

As described in the FlowChart, Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

This is performed in awake animals and typically takes up to 60 minutes, including the time to transport the animal. This procedure is typically performed in awake animals who are restrained in a primate chair, as described above, and head fixed for the safety of the animal and researcher. In some cases, the chamber will be cleaned while the animal is anesthetized (e.g. on days when a chamber is due for cleaning and the animal happens to be in surgery).

Procedures

1.	**Procedure Type:***	Training and Task Performance
2.	**Brief Description:***	Behavioral responses to stimuli
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	C

* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

During training and physiological procedures, monkeys are placed in their chairs in front of a computer monitor or projector screen. Their eye position is monitored and recorded using a video camera. Monkeys experience various video-game-like behavioral tasks and situations. For example, some of these include visual scenes in which monkeys learn to associate fixating a small target with liquid reward deliveries. In other tasks, visual images may instruct to perform a particular action to get a reward, such as using an eye movement or hand movement made with a lever or joystick. To motivate the animals, they are maintained on controlled water intake schedules that are individually tailored for each monkey.

Non-visual stimuli
Sometimes auditory stimuli are used, for example to indicate an error made by the monkey during a trial. All sound exposure will be less than 85 dB.

We will carefully observe animals by CCTV while they are working.

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

Training or recording sessions will be terminated if an animal exhibits overt signs suggestive of discomfort, pain or distress including grimacing, squirming, vocalization.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

During the task, behavioral assays (pupils, trial initiation time, and other measures of motivation) will be used,along with clinical observation on CCTV to determine if any problems arise.

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

As described in the FlowChart, Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Task performance will continue until the animal is no longer interested in working for fluid rewards. Working sessions may last up to 6 hours.

Procedures

1.	**Procedure Type:***	Behavioral or Physiological Testing
2.	**Brief Description:***	Electrophysiological Recording
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	C

* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

The electrical activity of individual neurons in the brain that discharge in relation to stimuli or related actions will be recorded to elucidate the underlying neural mechanisms. Neuronal responses will be recorded in various regions of the brain by introducing microelectrodes through the dura or through the artificial dura. These may be fine metal or glass microelectrodes, or multielectrode probes such as V-probes or silicon multielectrodes (e.g. Plexon, ATLAS, Neuropixels). For flexible electrodes such as tungsten microelectrodes, a guide tube is typically necessary to avoid bending of the probe when it passes the (artificial) dura. All guide tubes and electrodes will be sterilized before use, as described below. Particular care will be taken to minimize neural damage during the placement of the guide tubes and electrodes. For that reason, in chambers with artificial dura, we place a sterile insert consisting of a metal ring and a perforated plastic cover, on top of the artificial dura during recording sessions, to minimize brain movements due to pulsations from heartbeat and breathing. On top of this insert, we often position a few sterile metal spacer rings, and then a threaded metal ring that screws in the chamber. This configuration provides gentle downward pressure during recording to reduce brain movements due to pulsation from heartbeat or breathing, thereby minimizing damage that such movements would cause when the electrode is inserted. In chambers without an artificial dura, a sterile grid is placed in the chamber, which secures the stainless steel guide tube.
Intracerebral electrodes do not generally give rise to any pain or discomfort. Since the electrodes are extremely delicate and electrically sensitive, they cannot be mechanically cleaned or steam autoclaved. Before recording the electrode will be sterilized using UV radiation, 70% alcohol or cetylcide or dilute sodium hypochlorite solution (10% bleach) followed by rinse with sterile water, following manufacter's instructions. After recording, the tip of the electrode will be washed to remove any debris, according to manufacturer's instructions, typically using water, enzymatic cleaner and alcohol. The electrode will be stored in closed containers when not in use. The electrode is re-sterilized before each use. The above procedures will also be employed with other implants that enter the brain (guide tubes, cannulas, stimulating electrodes). Any device that is implanted in the brain, like an electrode or a guide-tube, will be removed from the individual animals should any complication(s) occur and DCM will be immediately consulted.

Electrical Stimulation
Stimulation through the recording electrode or through separately placed electrodes will be done to aid in the assessment of the functional organization of the recording region. Electric currents will be passed through the electrodes as pulses of short durations (0.1-1.0 milliseconds). The amount of currents will be less than a few milliamps (typically less than 200 microamps). Based on previous experience in various NHP labs, including in other labs at WUSM such as the Monosov lab, this procedure is rarely stressful.

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Anatomical marking
To facilitate unequivocal histological localization of the microelectrode recordings, small electrolytic lesions may be made by passing very small currents (10-20 microamps) for 10-30 s through pre-selected contacts of the recording probe. This procedure has been demonstrated in past experience to be non-stressful and will be done while the animal is awake since correct placement of the electrode can only be accomplished after first recording the neuronal activity associated with behavior; however, if there are signs of discomfort then general anesthesia will be employed. It may take 1-2 months before the lesion is visible so this procedure is typically performed 1-3 months before euthanasia. However, it may be performed up to 1 year prior to euthanasia as we do not see adverse clinical signs in response to the lesion.

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

If the monkey responds to the stimulation in a manner suggestive of discomfort (e.g., grimacing, vocalization, squirming, etc.) then the stimulation at that site will be immediately discontinued.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Since there are no nociceptive receptors in the brain, electrodes and needles cause no pain.
Since we typically use recording chambers in which the native dura in the chamber has been replaced by a silicone artificial dura, there is also no manipulation of the native dura during recording.
For recording chambers without an artificial dura, insertion of guide tubes through the dura could, in principle, cause brief, minor pain. This pain is apparently of less intensity than that produced by a hypodermic syringe inserted through the skin, as evidenced by the fact that the animals rarely, if ever, show a reaction when the electrode/guide tube is inserted. For animals that show signs of discomfort to the electrode/guide tube insertion, we will apply a drop or two of lidocaine (a local anesthetic) to the dura for a few minutes before insertion.
During the session behavioral assays (pupils, trial initiation time,and other measures of motivation) will be used,along with clinical observation on CCTV to determine if any problems arise.

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

As described in the FlowChart, Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Up to 8 hours, including cleaning of the chamber.

---

* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

Basic Covered Species Monitoring

Body Temperature, Respiratory Rate, etc.

Other with Specialized Equipment (Describe below)

Procedures

1.	**Procedure Type:***	Behavioral or Physiological Testing
2.	**Brief Description:***	Optogenetics
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	C

Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

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* * * Procedure Description * * *

Procedure Description

1. Detailed Procedure Description

Our physiological recordings coupled with psychophysical performance measures enable us to correlate behavior and underlying neuronal responses. Such measures are correlative, thus they cannot establish a causal link between neuronal activity and behavior. While electrical stimulation can be used to activate a small population of neurons near the stimulating electrode, such stimulation is non-selective (activating different types of neurons near the electrode tip), and causes an electrical artifact that interferes with our ability to record neuronal activity at the time of stimulation. Therefore, in some projects we will use optogenetic techniques, which involve the use of viruses to deliver DNA to neurons such that they express light-sensitive proteins (opsins), which, when stimulated with light of an appropriate frequency, will either hyperpolarize or depolarize the neurons that express these opsins. In addition, viral tropism and/or cell-type specific promoters can be used to ensure these opsins are expressed selectively within a specific subpopulation of neurons. Thus, using optogenetic techniques we can activate or inactivate neuronal subtypes. Because this involves delivery of light, it does not cause the electrical artifact that is introduced by traditional microstimulation techniques. Light will be delivered using a laser or LED system, via a fiber-optic cable. Irradiance will be measured, and will not exceed 250 milliwatts / square millimeter. The fiber-optic cable and cannula will be sterilized prior to the procedure (using either UV radiation, gas sterilization, cetylcide or 10% bleach followed by rinsing with sterile saline). Importantly, when the transduced region of cortex is superficial (located on the convexity of the brain), our recording chambers with transparent artificial dura often allow us to position the fiber-optic cable above the artificial dura, i.e. it does not need to be inserted into the brain if light delivery through the transparent artificial dura is sufficient. In cases where we do need to insert the fiber optic (100-200 microns in diameter) into the brain (e.g. deep targets), we will use a combination of a microelectrode or laminar probe for recording neuronal activity and electrical stimulation and a fiber optic in the procedures that involve inactivation and activation of neurons. This is exactly comparable to what other NHP laboratories at WUSM currently do, either for optogenetics, or in using a combination of an electrode and a tube for the injection of neuroactive chemicals.

Injection of replication incompetent virus
Injection of adeno-associated virus (AAV) carrying genes encoding for proteins such as opsins is often done under anesthesia, but will sometimes be conducted in the laboratory room while the monkey sits head-restrained in the chair, as during training and physiological recording. Indeed, we will sometimes need to perform viral injections while the animal is awake, for experiments where we actively need to map the brain region to be stimulated or silenced. A small guide tube may be used to introduce the micropipette or syringe needle carrying the virus into the brain. Syringe needle, micropipettes and guide tubes will be sterilized using appropriate methods (UV radiation, 10% bleach or cetylcide followed by sterile saline). The micropipette or syringe needle will be lowered into the brain, and then virus will be injected at a slow rate (e.g., 1 microliter every 5 minutes). The micropipette or syringe needle will then be withdrawn at a very slow rate. After the injection the monkey is returned to its home cage.

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

Insertion of guide tubes through the dura could, cause brief, minor pain. Animals rarely, if ever, show a reaction when the electrode/guide tube is inserted. In chambers with an artificial dura, there is no penetration of the native dura. No complications are expected.

3. Describe post procedure monitoring, observation schedules, and treatment that will be performed.

During the procedure behavioral assays (pupils, trial initiation time,and other measures of motivation) will be used,along with clinical observation to determine if any problems arise.

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

As described in the flowchart, sequence and timing section.

5. What is the duration of the procedure, from anesthesia to wake up?

Recording sessions that involve optogenetics can last up to 8 hours.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

Basic Covered Species Monitoring

Body Temperature, Respiratory Rate, etc.

Other with Specialized Equipment (Describe below)

Procedures

1.	**Procedure Type:***	Imaging of Anesthetized Animals
2.	**Brief Description:***	MRI of aneshetized animals
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	C

* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

MRI scans will be conducted in a Siemens Magnetom Vision scanner (1.5 or 3.0 Tesla) using a head coil designed for nonhuman primates or a surface coil.
The MRI scans will be done while the animal is anesthetized using Isoflurane/O2. The animal will be fasted for between 8-12 hours prior to anesthesia. The animal will initially be anesthetized with ketamine (10-15 mg/kg), atropine and metoclopramide if needed. In some cases the ketamine dose may be 3-5 mg/kg if used in conjunction with Dexmedetomidine at a dose of 0.01-0.03 mg/kg IM. It will be transported to the MRI scanner in the East Building. Once there, an endotracheal tube will be inserted and general anesthesia will be maintained via isoflurane for the duration of the MRI only scanning session. In some cases the inhalation anesthesia may be a combination of Isoflurane (1-5%) and Nitrous Oxide (20-75%) during scanning session. This combination of N2O/oxygen/Isoflurane reduces the hypotensive effects of anesthesia. The animal may be given Atipamezole 0.1-0.2 mg/kg IM to reverse the Dexmedetomidine, if it was given previously. To aid in the imaging of target sites, some monkeys will be injected intravenously with Magnevist (gadolinium, 0.2 mM/kg) during the scanning session. A small volume of gadolinium may also be put in a special designed cap(s) that covers the recording chamber(s). Each scan session typically lasts for 2-3 hours, after which time the animal is transferred back to the home cage where it is monitored until it regains a sitting posture.
We anticipate no more than 2 scans per week separated by 3 days at least for individual animals. Veterinary staff may cancel scheduled upcoming scan(s) if deemed necessary for the health and well-being of individual animals.

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

Any anesthesia session include the risk of adverse responses to anesthesia.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Animals will be continuously monitored until they have recovered from anesthesia.

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

As described in the Flowchart Sequence and Timing section.

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5. **What is the duration of the procedure, from anesthesia to wake up?**

The overall duration of the procedure is estimated to be 1-5 hours for anatomical scans.

Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Continuous pulse oximetry will be installed during all MRI imaging sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	4-5% induction, 1-3% maintenance	up to 4 hours	continuous
Nitrous Oxide	Inhalation (IN)	20-75% in combination with isoflurane	up to 4 hours	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg	works up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 4 hours	once in combination with low dose ketamine (3-5 mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	up to 4 hours
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	4-5% induction, 1-3% maintenance
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)

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3.	Duration of injections or administrations (if using inhalation agent)	up to 4 hours
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-75% in combination with isoflurane
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 4 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5 mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	

Other premedications not already listed above

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg		once to prevent excessive salivation
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg	once per procedure	once to prevent excessive salivation

Other premedications not already listed above

1.	Agent Name*	Atropine

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2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg to reverse the dexmedetomidine	given after removed from inhalation agent	once

Other Drugs Utilized

1.	Agent Name*	Other atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	given after removed from inhalation agent
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg to reverse the dexmedetomidine
6.	Volume of injections or administrations (where applicable)	
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Imaging of Anesthetized Animals
2.	Brief Description:*	CT scans
3.	Species: *	Non-Human Primate (various)
4.	USDA Pain/Distress Category:*	C

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* * * Procedure Description * * *

Procedure Description

1. Detailed Procedure Description

These scans will be performed outside of normal business hours since this will be done in (b) (4) . Scans will be coordinated with DCM veterinary staff since they will need to accompany us from intubation procedure before the scan (b) (4) (b) (4) until we are back inside (b) (4) after the scan. There is a protocol in place at (b) (4) from large animal scans that lists the cleaning products to use after the scan is complete and the animal is back on the DCM anesthesia cart for transport. (b) (4) lab staff will make sure this is followed before leaving the CT scan area.
The animal will be sedated with ketamine and atropine or glycopyrrolate and metoclopramide if needed. The animal will be intubated and anesthetized with isoflurane /oxygen in (b) (4) . DCM veterinary staff will accompany the lab staff and animal to the (b) (4) to CT scan imaging area, remain for

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the duration of the scan and back to [redacted] The animal will be transported on an anesthesia cart under general anesthesia with a portable pulse oximeter to monitor pulse and hemoglobin oxygen saturation (SpO2) to and from [redacted] area.

We anticipate that we need at most 1 CT scan per year for each animal. If there would be a need for multiple scans, a minimum recovery period of 2 weeks will be observed between scans.

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

This is a non-surgical procedure, includes little risk and is routinely done on human patients at high frequency with no complications. If an animal has an adverse reaction to anesthesia, DCM veterinary staff will be present to provide assistance and treatment.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

DCM staff will monitor during CT scan and transportation back to [redacted] Lab staff will be responsible for anesthesia recovery monitoring once back in [redacted] as is the case when recovering from general anesthesia from surgery and MRI scans. The animals will be recovered in the non-human primate ante-room with a member of DCM veterinary staff until extubated and then observed by lab staff in the animal's home cage. The animal will be observed by lab staff until the animal is awake and lifting head on its own. It will then be monitored at a minimum of every 15 minutes until it is sitting and awake and QAR (quiet,alert and responsive).

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

As described in the Flowchart Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Up to 1 hour

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

Other with Specialized Equipment (Describe below)

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once, if additional doses are required a lower dose will be used.

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.

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5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg		once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg	once per procedure	once to prevent excessive salivation
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID

Other premedications not already listed above

1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	administered with ketamine

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)

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3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Surgery
2.	Brief Description:*	Head post implantation
3.	Species: *	Non-Human Primate (various)

Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

4.	USDA Pain/Distress Category:*	D

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* * * Surgery Info * * *

Surgery Information

1. **Surgery Type:** S-Survival
2. **Surgery Classification** Major

NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

The purpose of the head post is to enable us to hold the head of the monkey rigid during the course of our experiments. This is achieved by connecting the head post during the recording session to a metal head bar that is connected to the primate chair. The head post implantation is generally the first procedure performed on each animal. Our head post design consists of 2 parts: a titanium bottom part that is implanted in the skull using bone screws (described below) and of which the top extends above the skin surface, and a metal top part (brass, stainless steel or titanium) that is connected to the top of the bottom part using dental acrylic.
Prior to surgery, the animal is deprived of food (but not water) for 8-12 hours. Prior to surgery, prophylactic antibiotics are given (Cefazolin 20-30 mg/kg IM BID or equivalent or/and as recommended by DCM veterinary staff; timing of first dose as recommended by DCM veterinary staff). Administration of the antiemetic Metoclopramide (Reglan) @ 0.2-0.3 mg/kg IM SID-BID will be used for animals that show a tendency to get nauseous or vomit either during or following the administration of anesthetic agents. Reglan will be given 10-20 minutes before preanesthetic injections. If nausea continues after recovery from anesthesia it can be given SID-TID as recommended by DCM veterinary staff. In addition, the DCM veterinary staff typically administers an analgesic(s) prior to beginning surgical procedures on the day of surgery. Examples of preoperative analgesics include Buprenorphine, Banamine, etc. Pre-emptive Buprenorphine dose is 0.01 mg/kg IM and @ 0.02-0.03 mg/kg IM every 8-10 hours postoperatively prn as determined by the DCM veterinary staff, or Buprenorphine SR 0.2 mg/kg SC will be administered prior to beginning the surgical procedure.

The animal is initially anesthetized with ketamine plus glycopyrrolate to reduce salivation. The animal is transferred to the surgical prep area, weighed, and the head is shaved and cleaned, followed by at least 3 scrubs with alcohol, betadine or Nolvasan. Eye lubricant will be applied. A sterile marker will be used to draw a line of where the incision line will be made and Lidocaine 1-2mg/kg mixed with Bupivicaine 1-2mg/kg will be injected ID along this line for local analgesia. An IV fluid drip (LRS) is begun (10ml/kg/hr, unless otherwise directed by the veterinarian). Surgical anesthesia is produced using isoflurane. The animal is intubated, connected to a respirator (typical initial isoflurane concentration: 4%) and the expired gas monitored by a CO2 meter. At this time, the animal is transferred to the surgery room. Once in the surgery room, the animal is mounted in a stereotaxic frame over a heating pad, and EKG electrodes, a rectal temperature probe and an oxygen saturation transducer (percentage of hemoglobin existing as oxyhemoglobin) are connected. Depth of anesthesia is monitored using heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (4–5%), and reflexes (e.g., toe pinch, corneal reflex). Supplemental heat will be provided such as Bair hugger or water recirculating blanket.

Once the animal is stable, the surgeon and (optional) assistant or trainee scrub and put on sterile gowns while the anesthetist continues to monitor the animal. Prior to starting surgery, a sterile drape will cover the animal and surgical table for instruments. Prior to the beginning of surgery all instruments, including implants, will be sterilized (heat, gas, liquid such as cetylcide for 20 minutes and then rinsed with sterile saline/water). We will start the surgery by making a straight rostrocaudal incision that is as small as possible, consistent with the need to access the underlying cranium to affix the head post to the cranium. The size of the incision will vary according to various factors, including the size of the skull and the looseness of the skin. Typically 2-3 inches. To aid healing, the incision is positioned 1-2 cm parasagittally. Instruments used for this step will include a sterile scalpel blade for incision, scissors for blunt dissection and incision, and forceps to hold the tissue. A periosteal elevator may be used to remove any soft tissue and the periosteum from the section of bone where the head post will be placed. In some animals there is a pronounced bone ridge on the skull. Part of this ridge may need to be shaved off to properly seat the implant. The titanium bottom part is attached to the calvarium using orthopedic screws (typically about 15 screws). In order to ensure that the screws do not protrude excessively through the inner cortices of the bone, the [redacted] lab at the [redacted] has examined a non-human primate calvarium post mortem, and determined that 6-8 mm screws are of a safe length that do not protrude excessively. Once the titanium bottom part is attached to the skull, the skin flap medial to the parasagittal incision is positioned over this part, and a small hole is made in this skin flap so that the top of this titanium bottom part can protrude through the skin. The skin is then sutured closed. We will use nonabsorbable suture material to close the skin (e.g. polypropylene, nylon). Sterile surgical staples are also often used to close the skin.

At this point, the surgery no longer requires sterility. A small quantity of dental cement or acrylic is then used to attach the metal top part to the top of the bottom part.

If bleeding occurs during the procedure, sterile gelfoam or similar products (with or without thrombin), and/or electrocautery may be used to stop bleeding. Gelfoam we use is freshly taken from a new sterile pack and we apply it under aseptic conditions so the risk of infection is low. Further, gelfoam is an absorbable material and therefore may be left under the skin, as long as an excessive amount is not used.

The animal recovers under observation until responsive and mobile and then is returned to its home cage. Prophylactic antibiotics (previously described) are continued for 3 days or as recommended by in-house veterinarian. Tylenol (10 mg/kg, PO, prn up to qid) or Buprenorphine SR (0.2mg/kg, SC once that lasts for up to 72 hours) or Buprenorphine (0.01-0.03 mg/kg, IM, bid/prn) is given as necessary over the next 2 days. Cefazolin is given IM post-operatively as suggested by DCM staff.

Non-resorbable sutures or staples will be removed after 10-14 days.

Potential reasons for multiple surgeries.
An acrylic repair may be needed if the acrylic connecting the top part of the head post to the base becomes loose. With a loose acrylic component, the integrity of the headpost implant is not sufficient to bear the force of head restraint and there is danger of it becoming traumatically dislodged. When such failure is suspected, the acrylic will be removed and new acrylic will be applied to

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fasten the top part of the head post to the base. This is a procedure under anesthesia but non-invasive (only the part of the headpost above the skin surface needs to be repaired).
Failure of the bottom of the implant may require the elective removal of the implant and re-closure of the skin over the skull defect to allow adequate time for bone healing before re-implanting another cephalic implant. Another possibility is to disinfect the region of the old implant, and to put acrylic on the skull. After approximately 1-2 weeks, a new implant may be attached. Which route we take, depends on what is best for the health of the animal and our research.

We will follow the IACUC Policy: Anesthesia, Surgery and Post Procedural Care for Non-Rodent Mammals for all survival surgical procedures.

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

Potential complication involves infections along wound margins or under the head post assembly. We take strong precautions to minimize the risk of infection and to eliminate or contain any infection if it does occur. Although rare, there is a finite risk that the head post assembly can loosen or break. On the rare occasions when this occurs, we immediately take corrective steps, which may include a surgical operation to remove or replace the particular part and repair the implant (as described above).
Alternatively, if the cephalic implant loosens or fails, it may be necessary to remove the implant (i.e., bone cement and bone screws) and primarily close the skin margins over the skull defect and allow a suitable period of time to allow for bone healing to occur before implanting a new cephalic implant.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

In addition to the routine inspection and cleaning of skin margins, we apply topical antibiotic ointment if there are signs of infection, and we consult closely with the veterinary staff about appropriate topical and/or systemic treatments to be used.

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

As described in the Flowchart Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Typically 4-6 hours.

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* * * Personnel Details * * *

Personnel Details

Personnel Details

Select Names of personnel who will be performing this surgical procedure for this protocol. * Check all that apply.
[redacted]

Personnel Details

1. Select Names of personnel who will be performing this surgical procedure for this protocol.* [redacted]
Check all that apply.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg		once to prevent excess salivation
Lactated Ringer's solution (LRS) drip	Intravenous (IV)	LRS	continuous during procedure	once
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID

Other premedications not already listed above

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1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excess salivation
5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other Lactated Ringer's solution (LRS) drip
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	continuous during procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	LRS
6.	Volume of injections or administrations (where applicable)	10 ml/kg/hr, unless otherwise directed by DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Antibiotics or Anti-Microbials

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Cefazolin	IM or IV	20-30 mg/kg	works for 8-12 h	prior to surgery then twice a day for 1-5 days

Antibiotics or Anti-Microbials

1.	Agent Name*	Cefazolin
2.	Route of Administration*	Other IM or IV
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 h
4.	Frequency of injections or administrations (if using inhalation agent)	prior to surgery then twice a day for 1-5 days
5.	Dose of injections or administrations	20-30 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls for IM

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours

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4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after procedure/surgery.	No additional monitoring after the first 4 days.
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	Animals will be monitored closely during initial recovery from anesthesia. After extubation, animals will be returned to their home cage where they will be continuously monitored until they are able to sit up on their own. Once they are sitting they will be monitored every 15-30 minutes for the hour after they are sitting up on their own. For the ensuing four hours, animals will continue to be examined regarding respiration rate, visible signs of activity and alertness, appetite, bleeding, swelling, overall appearance, balance, use of limbs, spontaneous head and eye movements, and signs of pain or discomfort at hourly intervals, and monitored at least twice a day by lab and/or DCM for the following 2 days. Responsiveness to visual and auditory stimulation will be monitored at frequent intervals during the first 2 days. Monitoring will continue at least once per day during day 3 and day 4, thus at least until 96 hours after surgery.

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Surgery
2.	Brief Description:*	Craniotomy and recording chamber implantation
3.	Species: *	Non-Human Primate (various)
4.	USDA Pain/Distress Category:*	D

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* * * Surgery Info * * *

Surgery Information

1.	Surgery Type:	S-Survival
2.	Surgery Classification	Major

NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.

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Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

The purpose of the recording chamber is to be able to use high-resolution electrophysiological or optical recording and/or perturbation techniques (e.g. electrode recordings, optogenetics, electrical stimulation, optical imaging - widefield or multiphoton) to study the brain circuits underlying complex perception.

Prior to surgery, the animal is deprived of food (but not water) for 8-12 hours. Prior to surgery, prophylactic antibiotics are given (Cefazolin 20-30 mg/kg IM BID or equivalent or/and as recommended by DCM veterinary staff; timing of first dose as recommended by DCM veterinary staff). Administration of the antiemetic Metoclopramide (Reglan) @ 0.2-0.3 mg/kg IM SID-BID will be used for animals that show a tendency to get nauseous or vomit either during or following the administration of anesthetic agents. Reglan will be given 10-20 minutes before preanesthetic injections. If nausea continues after recovery from anesthesia it can be given SID-TID as recommended by DCM veterinary staff. In addition, the DCM veterinary staff typically administers an analgesic(s) prior to beginning surgical procedures on the day of surgery. Examples of preoperative analgesics include Buprenorphine, Banamine, etc. Pre-emptive Buprenorphine dose is 0.01 mg/kg IM and @ 0.02-0.03 mg/kg IM every 8-10 hours postoperatively prn as determined by the DCM veterinary staff, or Buprenorphine SR 0.2 mg/kg SC will be administered prior to beginning the surgical procedure.

The animal is initially anesthetized with ketamine plus glycopyrrolate to reduce salivation. The animal is transferred to the surgical prep area, weighed, and the surgical site is shaved and cleaned, followed by at least 3 scrubs with alcohol, betadine or Nolvasan. Eye lubricant will be applied. A sterile marker will be used to draw a line of where the incision line will be made and Lidocaine 1-2mg/kg mixed with Bupivicaine 1-2mg/kg will be injected ID along this line for local analgesia. IV fluids (LRS) will be administered at a rate of 10ml/kg/hr, unless otherwise directed by the veterinarian). Surgical anesthesia is produced using isoflurane. The animal is intubated, connected to a respirator (typical initial isoflurane concentration: 4%) and the expired gas monitored by a CO2 meter. At this time, the animal is transferred to the surgery room. Once in the surgery room, the animal is mounted in a stereotaxic frame over a heating pad, and EKG electrodes, a rectal temperature probe and an oxygen saturation transducer (percentage of hemoglobin existing as oxyhemoglobin) are connected. Depth of anesthesia is monitored using heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (4–5%), and reflexes (e.g., toe pinch, corneal reflex). Supplemental heat will be provided such as Bair hugger or water recirculating blanket.

Once the animal is stable, the surgeon and (optional) assistant or trainee scrub and put on sterile gowns while the anesthetist continues to monitor the animal. Prior to starting surgery, a sterile drape will cover the animal and surgical table for instruments. Prior to the beginning of surgery all instruments, including implants, will be sterilized (heat, gas, liquid such as cetylcide for 20 minutes and then rinsed with sterile saline/water). Recording chamber implantation involves the attachment of plastic or metal chamber(s) to the skull. Typically either one or two chamber(s) are implanted. They are attached to the skull using sterile orthopedic screws and a small quantity of bone cement or dental acrylic. The purpose of each chamber is to provide a sealable "window" that allows direct access to the tissue that lies at the bottom of the chamber. It is through this window that we can later insert microelectrodes during the course of our electrophysiological experiments, or through which light can be delivered for optogenetics experiments or transmitted for optical imaging experiments. The chambers are kept sealed when not in use, and are regularly cleaned. There are three versions of the procedure, as follows: Procedure A: a craniotomy is placed; a chamber is then lowered into the craniotomy and is affixed to the cranium using screws and dental acrylic or bone cement. Procedure B: a chamber is affixed to the cranium using screws and dental acrylic or bone cement; in a separate surgery, a craniotomy is made within the chamber. Procedure B may be used when placing two chambers, in order to limit time on table by delaying the craniotomy surgery until a second surgery. It may also be used if the surgery is long in duration, to avoid excessive time on table. Prior to closing the chamber, we apply a silicone seal between the cap and the chamber to further reduce risk of infection. After closing the chamber, melted bone wax is typically applied on the edge between cap and chamber to further reduce the risk of infection.

If bleeding occurs during the procedure, sterile gelfoam or similar products (with or without thrombin), and/or electrocautery may be used to stop bleeding. Gelfoam we use is freshly taken from a new sterile pack and we apply it under aseptic conditions so the risk of infection is low. Further, gelfoam is an absorbable material and therefore may be left under the skin, as long as an excessive amount is not used.
At the end of surgery, the skin and tissue will be sutured. The most common suture type used is sterile PDS. Sterile surgical staples are also often used to close the skin.

The animal recovers under observation until responsive and mobile and then is returned to its home cage. Prophylactic antibiotics (previously described) are continued for 3 days or as recommended by in-house veterinarian. Tylenol (10 mg/kg, PO, prn up to qid) or Buprenorphine SR (0.2mg/kg, SC once that lasts for up to 72 hours) or (buprenorphine) (0.01-0.03 mg/kg, IM, bid/prn) is given as necessary over the next 2 days. Cefazolin is given IM post-operatively as suggested by DCM staff.

Non-resorbable sutures or staples will be removed after 10-14 days.

Potential reasons for multiple surgeries.
This procedure will often be followed by a separate procedure, typically 2 weeks later, in which a durotomy is performed in the chamber to install an artificial dura. This is necessary to be able to precisely position thin multielectrode probes orthogonal to the cortical surface while avoiding damage to blood vessels and/or to deliver light through the artificial dura for optogenetics, or for optical imaging. Often, typically another 2 weeks later, viral injections are performed in the brain tissue in the chamber to express proteins (e.g. opsins for optogenetics or calcium indicators for imaging).
If no further recordings from the brain tissue in a chamber are necessary, the chamber is often removed, to contribute to the animal's

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comfort by reducing the number of cleaning sessions.

We will follow the IACUC Policy: Anesthesia, Surgery and Post Procedural Care for Non-Rodent Mammals for all survival surgical procedures.

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

Potential complication involves infections along wound margins or under the implant assembly. We take strong precautions to minimize the risk of infection and to eliminate or contain any infection if it does occur. Failure of a cephalic implant may require the elective removal of the implant and re-closure of the skin over the skull defect to allow adequate time for bone healing before re-implanting another cephalic implant. Another possibility is to disinfect the region of the old implant, and to put acrylic on the skull. After approximately 1-2 weeks, a new implant may be attached. Which route we take, depends on what is best for the health of the animal and our research.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

In addition to the routine inspection and cleaning of skin margins, we apply topical antibiotic ointment if there are signs of infection, and we consult closely with the veterinary staff about appropriate topical and/or systemic treatments to be used.

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

As described in the Flowchart Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Typically 5-7 hours of the animal under anesthesia, depending upon the number of chambers implanted.

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* * * Personnel Details * * *

Personnel Details

Personnel Details

Select Names of personnel who will be performing this surgical procedure for this protocol. * Check all that apply.
[redacted]

Personnel Details

1. Select Names of personnel who will be performing this surgical procedure for this protocol.* [redacted]
Check all that apply.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg		once to prevent excess salivation
LRS drip	Intravenous (IV)	LRS	continuous during procedure	once
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID

Other premedications not already listed above

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1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excess salivation
5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other LRS drip
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	continuous during procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	LRS
6.	Volume of injections or administrations (where applicable)	10ml/kg/hr, unless otherwise directed by DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Antibiotics or Anti-Microbials

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Cefazolin	IM or IV	20-30 mg/kg	works for 8-12 h	prior to surgery then twice a day for 1-5 days

Antibiotics or Anti-Microbials

1.	Agent Name*	Cefazolin
2.	Route of Administration*	Other IM or IV
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 h
4.	Frequency of injections or administrations (if using inhalation agent)	prior to surgery then twice a day for 1-5 days
5.	Dose of injections or administrations	20-30 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls for IM

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours

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4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after procedure/surgery.	No additional monitoring after the first 4 days.
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	Animals will be monitored closely during initial recovery from anesthesia. After extubation, animals will be returned to their home cage where they will be continuously monitored until they are able to sit up on their own. Once they are sitting they will be monitored every 15-30 minutes for the hour after they are sitting up on their own. For the ensuing four hours, animals will continue to be examined regarding respiration rate, visible signs of activity and alertness, appetite, bleeding, swelling, overall appearance, balance, use of limbs, spontaneous head and eye movements, and signs of pain or discomfort at hourly intervals, and monitored at least twice a day by lab and/or DCM for the following 2 days. Responsiveness to visual and auditory stimulation will be monitored at frequent intervals during the first 2 days. Monitoring will continue at least once per day during day 3 and day 4, thus at least until 96 hours after surgery.

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Surgery
2.	Brief Description:*	Durotomy and installing artificial dura
3.	Species: *	Non-Human Primate (various)
4.	USDA Pain/Distress Category:*	D

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* * * Surgery Info * * *

Surgery Information

1.	Surgery Type:	S-Survival
2.	Surgery Classification	Major

NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.

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Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

* * * Procedure Description * * *

Procedure Description

1. Detailed Procedure Description

The purpose of the durotomy and installation of an artificial dura is to obtain optical access to the surface of the cortical tissue, because the dura in this species is opaque. This allows us to precisely inject viral vectors in selected locations; insert thin multielectrode probes precisely and orthogonally relative to the cortical surface; deliver light through the transparent artificial dura for optogenetics studies. This procedure will follow a craniotomy and chamber implantation. To avoid long procedures, it occurs typically as a separate procedure, typically 2 weeks after craniotomy/chamber implantation.

Prior to surgery, the animal is deprived of food (but not water) for 8-12 hours. Prior to surgery, prophylactic antibiotics are given (Cefazolin 20-30 mg/kg IM BID or equivalent or/and as recommended by DCM veterinary staff; timing of first dose as recommended by DCM veterinary staff). Administration of the antiemetic Metoclopramide (Reglan) @ 0.2-0.3 mg/kg IM SID-BID will be used for animals that show a tendency to get nauseous or vomit either during or following the administration of anesthetic agents. Reglan will be given 10-20 minutes before preanesthetic injections. If nausea continues after recovery from anesthesia it can be given SID-TID as recommended by DCM veterinary staff. In addition, the DCM veterinary staff typically administers an analgesic(s) prior to beginning surgical procedures on the day of surgery. Examples of preoperative analgesics include Buprenorphine, Banamine, etc. Pre-emptive Buprenorphine dose is 0.01 mg/kg IM and @ 0.02-0.03 mg/kg IM every 8-10 hours postoperatively prn as determined by the DCM veterinary staff, or Buprenorphine SR 0.2 mg/kg SC will be administered prior to beginning the surgical procedure. To prevent brain edema we will typically administer Dexamethasone and mannitol. A dose of 0.05-2 mg/kg of Dexamethasone may be given the night before, a dose of 0.05-2 mg/kg the day of surgery and then reduced over time according to animal health in consultation with DCM veterinarians. Mannitol will be administered on the day of surgery, at least 30 minutes prior to opening the dura mater (0.5-2.2 g/kg IV, over 20-60 minutes).
The use of mannitol as described here is specific to prevent brain edema in procedures that require installation of an artificial dura over the cortex. In these procedures, it is especially important to avoid any damage to the cortical surface, and therefore to avoid any swelling of the brain. Otherwise the cortical surface may become damaged during the durotomy, or during the installation of brim of the artificial dura underneath the dural edge, which would prevent obtaining electrophysiological or optical data from this part of the cortex. It is thus the standard approach of laboratories that use this procedure to administer mannitol at least 30 minutes prior to opening the dura, because mannitol takes time to start working. This approach is used in all labs doing such procedures in macaque monkeys as far as we know, including the (b) (4) (b) (6); (b)(7)(C) the (b) (4) the (b) (4) the (b) (4) the (b) (4) with excellent outcomes. This approach has been published in Shtoyerman et al., J Neurosci 2000 doi 10.1523/JNEUROSCI.20-21-08111.2000 (b) (4)

The animal is initially anesthetized with ketamine plus glycopyrrolate to reduce salivation. The animal is transferred to the surgical prep area, weighed, and the surgical site is shaved and cleaned, followed by at least 3 scrubs with alcohol, betadine or Nolvasan. Eye lubricant will be applied. IV fluids (LRS) will be administered at a rate of 10ml/kg/hr, unless otherwise directed by the veterinarian). Surgical anesthesia is produced using isoflurane. The animal is intubated, connected to a respirator (typical initial isoflurane concentration: 4%) and the expired gas monitored by a CO2 meter. At this time, the animal is transferred to the surgery room. Once in the surgery room, the animal is mounted in a stereotaxic frame over a heating pad, and EKG electrodes, a rectal temperature probe and an oxygen saturation transducer (percentage of hemoglobin existing as oxyhemoglobin) are connected. Depth of anesthesia is monitored using heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex). To prevent brain edema, hyperventilation may be induced during the surgery, and monitored by expired CO2, in consultation with the DCM veterinarian. Supplemental heat will be provided such as Bair hugger or water recirculating blanket.

Once the animal is stable, the surgeon and (optional) assistant or trainee scrub and put on sterile gowns while the anesthetist continues to monitor the animal. Prior to starting surgery, a sterile drape will cover the animal and surgical table for instruments. Prior to the beginning of surgery all instruments, including implants, will be sterilized (heat, gas, liquid such as cetylcide for 20 minutes and then rinsed with sterile saline/water).

We will remove the native dura in the chamber and replace it with an artificial dura. The artificial dura will be made of polyurethane Tecoflex, silicone Polydimethylsiloxane (General Electric), or a similar material. Before surgery, the artificial dura will be sterilized. To keep the artificial dura in place, the artificial dura is in the form of a "hat". The flange of the artificial dura (the brim of the hat) will be inserted under the native dura, and the cylinder will protrude from the durotomy.
To prevent infection, a small volume of antibiotic [either Amikacin (0.1-0.3 ml, 250mg/ml) or Gentamicin (0.1-0.3 ml, 50mg/ml)] is placed on a small piece of sterile gauze that is positioned on top of the artificial dura.

If bleeding occurs during the procedure, sterile gelfoam or similar products (with or without thrombin), and/or electrocautery may be used to stop bleeding. Gelfoam we use is freshly taken from a new sterile pack and we apply it under aseptic conditions so the risk of infection is low. Further, gelfoam is an absorbable material and therefore may be left under the skin, as long as an excessive amount is not used.

The animal recovers under observation until responsive and mobile and then is returned to its home cage. Prophylactic antibiotics (previously described) are continued for 3 days or as recommended by in–house veterinarian. Tylenol (10 mg/kg, PO, prn up to qid) or Buprenorphine SR (0.2mg/kg, SC once that lasts for up to 72 hours) or Buprenorphine (0.01-0.03 mg/kg, IM, bid/prn) is given as necessary over the next 2 days. Cefazolin is given IM post-operatively as suggested by DCM staff.

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Potential reasons for multiple surgeries.
This procedure will often be preceded by a separate procedure, typically 2 weeks earlier, in which a craniotomy and recording chamber has been installed. It will often be followed by a separate procedure, typically 2 weeks later, in which viral injections in the recording chamber are carried out. When possible we seek to minimize the total number of surgeries by combining two of these procedures into a single surgery. However, we must balance this against the risks associated with long surgeries (duration varies, e.g. depending on the number of sites that will be injected with virus). See Flowchart, sequence and timing section.

We will follow the IACUC Policy: Anesthesia, Surgery and Post Procedural Care for Non-Rodent Mammals for all survival surgical procedures.

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

Potential complications include infection along wound margins or under the artificial dura. We take strong precautions to minimize the risk of infection and to eliminate or contain any infection if it does occur. Using the procedure described above, the [redacted] at the [redacted] has not seen this procedure to be complicated by infection. If infection should occur, this may require the elective removal of the implant. Systemic and/or local antibiotic treatment will be started in consultation with the DCM veterinarian. Another possible complication is postoperative bleeding under the dura/artificial dura. We minimize the risk by proper hemostasis during the surgery as described above. Often a small amount of blood is observed under the artificial dura during the first chamber cleaning, which doesn't require any treatment and disappears within a few weeks. Should there be a substantial amount of blood that exerts pressure on the underlying tissue, we will start appropriate treatment in consultation with the DCM veterinarian. This may include elective replacement of the artificial dura so that the clot can be removed.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

As described in section Routine Maintenance of Recording Chamber With Artificial Dura', recording chambers with artificial dura are cleaned at least every 5 days. During cleanings routine inspection of the wound margins and the chamber occurs, as well as of the tissue underneath the transparent artificial dura. In durotomies without a recording chamber, standard post procedure monitoring of wound margins and clinical status of the animal will allow us to detect complications promptly, and start the appropriate treatment in consultation with the DCM veterinarian.

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

As described in the Flowchart Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Typically 4-7 hours of the animal under anesthesia. While the placement of the artificial dura (the final step of the procedure before closing the chamber) doesn't take long, performing the durotomy can take a while. The durotomy needs to be performed carefully to avoid any damage to the cortical surface. This is important because damage to the surface can prevent obtaining optical or electrophysiological data. Furthermore, the native dura in the chamber needs be removed close to the circumference of the chamber (to allow proper placement of the artificial dura), which takes time. Factors that can increase the duration of the durotomy include the degree of brain edema that is encountered, presence of small blood vessels in the dura and CSF buildup in the chamber.

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* * * Personnel Details * * *

Personnel Details

Personnel Details

Select Names of personnel who will be performing this surgical procedure for this protocol. * Check all that apply.
[redacted]

Personnel Details

1. Select Names of personnel who will be performing this surgical [redacted] procedure for this protocol.*
Check all that apply.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

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Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg		once to prevent excess salivation
LRS drip	Intravenous (IV)	LRS	continuous during procedure	once
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID
Dexamethasone	Intramuscularly (IM)	0.5 mg/kg on the day before surgery; 1-2 mg/kg on the day of surgery, and then reduced over time over the next days according to animal health in consultation with DCM veterinarian		SID-BID
Mannitol	Intravenous (IV)	0.5-2.2 g/kg	over 20-60 minutes	dose may be repeated depending on intracranial swelling, in consultation with DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excess salivation

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5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other LRS drip
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	continuous during procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	LRS
6.	Volume of injections or administrations (where applicable)	10ml/kg/hr, unless otherwise directed by DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

Other premedications not already listed above

1.	Agent Name*	Dexamethasone
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.5 mg/kg on the day before surgery; 1-2 mg/kg on the day of surgery, and then reduced over time over the next days according to animal health in consultation with DCM veterinarian
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other Mannitol
2.	Route of Administration*	Intravenous (IV)

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3.	Duration of injections or administrations (if using inhalation agent)	over 20-60 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	dose may be repeated depending on intracranial swelling, in consultation with DCM veterinarian
5.	Dose of injections or administrations	0.5-2.2 g/kg
6.	Volume of injections or administrations (where applicable)	typically less than 100 mL

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Antibiotics or Anti-Microbials

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Cefazolin	IM or IV	20-30 mg/kg	works for 8-12 h	prior to surgery then twice a day for 1-5 days
Amikacin	Topical (topical)	0.1-0.3 mL, 250 mg/ml	small volume left in recording chamber at end of procedure	once during procedure. Repeated during chamber cleanings.
Gentamicin	Topical (topical)	0.1-0.3 mL, 50 mg/ml	small volume left in recording chamber at end of procedure	once during procedure. Repeated during chamber cleanings.

Antibiotics or Anti-Microbials

1.	Agent Name*	Cefazolin
2.	Route of Administration*	Other IM or IV
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 h
4.	Frequency of injections or administrations (if using inhalation agent)	prior to surgery then twice a day for 1-5 days
5.	Dose of injections or administrations	20-30 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls for IM

Antibiotics or Anti-Microbials

1.	Agent Name*	Other Amikacin
2.	Route of Administration*	Topical (topical)
3.	Duration of injections or administrations (if using inhalation agent)	small volume left in recording chamber at end of procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once during procedure. Repeated during chamber cleanings.
5.	Dose of injections or administrations	0.1-0.3 mL, 250 mg/ml
6.	Volume of injections or administrations (where applicable)	0.1-0.3 mL

Antibiotics or Anti-Microbials

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1.	Agent Name*	Gentamicin
2.	Route of Administration*	Topical (topical)
3.	Duration of injections or administrations (if using inhalation agent)	small volume left in recording chamber at end of procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once during procedure. Repeated during chamber cleanings.
5.	Dose of injections or administrations	0.1-0.3 mL, 50 mg/ml
6.	Volume of injections or administrations (where applicable)	0.1-0.3 mL

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after	No additional monitoring after the first 4 days.

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	procedure/surgery.	
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	Animals will be monitored closely during initial recovery from anesthesia. After extubation, animals will be returned to their home cage where they will be continuously monitored until they are able to sit up on their own. Once they are sitting they will be monitored every 15-30 minutes for the hour after they are sitting up on their own. For the ensuing four hours, animals will continue to be examined regarding respiration rate, visible signs of activity and alertness, appetite, bleeding, swelling, overall appearance, balance, use of limbs, spontaneous head and eye movements, and signs of pain or discomfort at hourly intervals, and monitored at least twice a day by lab and/or DCM for the following 2 days. Responsiveness to visual and auditory stimulation will be monitored at frequent intervals during the first 2 days. Monitoring will continue at least once per day during day 3 and day 4, thus at least until 96 hours after surgery.

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Non-Surgical Procedure Under Anesthesia

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2.	**Brief Description:***	Viral injections in a recording chamber
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	D

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* * * Procedure Description * * *

Procedure Description

1. Detailed Procedure Description

The purpose of viral injections in the brain is to express certain proteins in targeted locations. Such proteins can be for example opsins for optogenetics studies, GCaMP for optical imaging of calcium signals, or fluorescent proteins for histology. This procedure describes viral injections in the tissue in a pre-existing chamber. This setting allows us to inject viral vectors in the brain, analog to injection of viral vectors or drugs routinely done in other NHP labs at WUSTL, in a non-surgical procedure under anesthesia.

Prior to the procedure, the animal is deprived of food (but not water) for 8-12 hours. Prior to the procedure, prophylactic antibiotics are given (Cefazolin 20-30 mg/kg IM BID or equivalent or/and as recommended by DCM veterinary staff; timing of first dose as recommended by DCM veterinary staff). Administration of the antiemetic Metoclopramide (Reglan) @ 0.2-0.3 mg/kg IM SID-BID will be used for animals that show a tendency to get nauseous or vomit either during or following the administration of anesthetic agents. Reglan will be given 10-20 minutes before preanesthetic injections. If nausea continues after recovery from anesthesia it can be given SID-TID as recommended by DCM veterinary staff. In addition, the DCM veterinary staff typically administers an analgesic(s) prior to beginning the procedure. Examples of analgesics include Buprenorphine, Banamine, etc. Pre-emptive Buprenorphine dose is 0.01 mg/kg IM and @ 0.02-0.03 mg/kg IM every 8-10 hours post-procedure prn as determined by the DCM veterinary staff, or Buprenorphine SR 0.2 mg/kg SC will be administered prior to beginning the procedure. To prevent brain edema we will typically administer Dexamethasone and mannitol. A dose of 0.05-2 mg/kg of Dexamethasone may be given the night before, a dose of 0.05-2 mg/kg the day of the procedure and then reduced over time according to animal health in consultation with DCM veterinarians. Mannitol will be administered on the day of procedure, at least 30 minutes prior to starting the injections (0.5-2.2 g/kg IV, over 20-60 minutes).
The use of mannitol as described here is specific to prevent brain edema in procedures that require installation of an artificial dura over the cortex. In these procedures, it is especially important to avoid any damage to the cortical surface, and therefore to avoid any swelling of the brain. Otherwise the cortical surface may become damaged during the durotomy, or during the installation of brim of the artificial dura underneath the dural edge, which would prevent obtaining electrophysiological or optical data from this part of the cortex. It is thus the standard approach of laboratories that use this procedure to administer mannitol at least 30 minutes prior to opening the dura, because mannitol takes time to start working. This approach is used in all labs doing such procedures in macaque monkeys as far as we know, including the (b) (4) lab at the (b) (4) (b) (6), (b) (7)(C) , the (b) (4) , the (b) (4) the (b) (4) the (b) (4) with excellent outcomes. This approach has been published in Shtoyerman et al., J Neurosci 2000 doi 10.1523/JNEUROSCI.20-21-08111.2000 (b) (4) .

The animal is initially anesthetized with ketamine plus glycopyrrolate to reduce salivation. The animal is transferred to the prep area, weighed, and the chamber site is shaved and cleaned, followed by at least 3 scrubs with alcohol, betadine or Nolvasan. Eye lubricant will be applied. IV fluids (LRS) will be administered at a rate of 10ml/kg/hr, unless otherwise directed by the veterinarian). Anesthesia is produced using isoflurane. The animal is intubated, connected to a respirator (typical initial isoflurane concentration: 4%) and the expired gas monitored by a CO2 meter. At this time, the animal is transferred to the surgery room. Once in the surgery room, the animal is mounted in a stereotaxic frame over a heating pad, and EKG electrodes, a rectal temperature probe and an oxygen saturation transducer (percentage of hemoglobin existing as oxyhemoglobin) are connected. Depth of anesthesia is monitored using heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex). To prevent brain edema, hyperventilation may be induced during the procedure, and monitored by expired CO2, in consultation with the DCM veterinarian. Supplemental heat will be provided such as Bair hugger or water recirculating blanket.

Once the animal is stable, the researcher leading the procedure and (optional) assistant or trainee scrub and put on sterile gowns while the anesthetist continues to monitor the animal. Prior to starting the procedure, a sterile drape will cover the animal and table for instruments.

We will inject virus into the brain tissue underlying the dura or the artificial dura. We will either 1) remove the artificial dura from over the brain tissue, make the injections directly into the exposed tissue, and replace with a new, sterilized artificial dura; or 2) make the injections through the transparent artificial dura or the native dura. The virus will be injected using 1) a calibrated sterile glass micropipette coupled to tubing and a syringe, with which pressure can be controlled manually during the injection (monitored with a manometer), or 2), a Hamilton syringe with a sterile pipette tip. Preventing damage of the tissue is of primary importance to us, and is in fact why we use these methods to deliver virus, when feasible, instead of an injectrode. A glass micropipette or Hamilton syringe filled with virus lowered into the brain. Micropipette or syringe are appropriately sterilized before use using either UV irradiation, 10% bleach or cetylcide followed by rinse with sterile saline. The viruses to be injected include the following recombinant, replication incompetent viruses: AAV or lentivirus. Pressure and volume of injected virus and depth of pipette are precisely controlled. The

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injection speed is 50-100 nanoliter/min. When selecting locations for the injections, we avoid key structures such as blood vessels. We carefully monitor the surface of the brain for swelling. Most importantly, histological analysis of brain tissue after injection of virus has shown very minimal damage to brain tissue, and clear protein expression, indicating healthy neurons at the injection site. The pipette will then be removed.
At the end of the procedure, a small piece of sterile gauze with topical antibiotic (0.1-0.3 mL Amikacin or Gentamicin) will be left in place on top of the artificial dura.

We will follow the IACUC Policy: Anesthesia, Surgery and Post Procedural Care for Non-Rodent Mammals for all survival surgical and non-surgical procedures under anesthesia.

The animal recovers under observation until responsive and mobile and then is returned to its home cage. Prophylactic antibiotics (previously described) are continued for 3 days or as recommended by in-house veterinarian. Tylenol (10 mg/kg, PO, prn up to qid) or Buprenorphine SR (0.2mg/kg, SC once that lasts for up to 72 hours) or Buprenorphine (0.01-0.03 mg/kg, IM, bid/prn) is given as necessary over the next 2 days. Cefazolin is given IM post-procedure as suggested by DCM staff.

Potential reasons for multiple viral injections.
There are experimental justifications for injections to be made at different times or repeated. If the length of time needed exceeds reasonable time for anesthetized animals, we may repeat the injection procedure to target additional sites in a separate session. Two other cases in which we may repeat the viral injection procedure are: 1. when we need to compare protein expression following injection of two candidate viral constructs. The scientific justification for this is that testing one virus in each of two separate animals would introduce a confound in which differences in protein expression patterns could be attributable to either differences in the virus or differences in protein expression across animals. To enable us to determine differences in protein expression across viruses, the injections need to be made in the same animal. Given the time required for injection of one virus, it may not be feasible to inject additional viruses in one session. A typical injection time for a single site is 5-15 minutes. We will try to keep the duration the animal is under anesthesia under seven hours though we may extend the procedure with veterinary approval, depending on animal health. 2. when we need to test protein expression patterns over two points in time. In this case, we inject the virus in one site, and then wait a period of time comparable to the time we anticipate using the virus in optogenetic activation experiments (in other animals) before injecting the virus in a second site. After time has passed to allow protein expression in the second site, we will euthanize the animal and perform histology to examine protein expression patterns at the two locations, corresponding to two different periods of protein expression.

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

Potential complications include bacterial infection . We take strong precautions to minimize the risk of infection and to eliminate or contain any bacterial infection if it does occur. Using the procedure described above, the [redacted] at the [redacted] has not seen this to occur. If it should occur, this may require the elective removal of the implant. Systemic and/or local antibiotic treatment will be started in consultation with the DCM veterinarian.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

As described in section Routine Maintenance of Recording Chamber With Artificial Dura', recording chambers with artificial dura are cleaned at least every 5 days. During cleanings routine inspection of the wound margins and the chamber occurs, as well as of the tissue underneath the transparent artificial dura. In durotomies without a recording chamber, standard post procedure monitoring of wound margins and clinical status of the animal will allow us to detect complications promptly, and start the appropriate treatment in consultation with the DCM veterinarian.

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

As described in the Flowchart Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Typically 5-7 hours of the animal under anesthesia.

Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

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Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg		once to prevent excess salivation
LRS drip	Intravenous (IV)	LRS	continuous during procedure	once
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID
Dexamethasone	Intramuscularly (IM)	0.05-2 mg/kg on the day before surgery; 0.05-2 mg/kg on the day of surgery, and then tapered over 3-7 days according to animal health in consultation with DCM veterinarian		SID-BID
Mannitol	Intravenous (IV)	0.5-2.2 g/kg	over 20-60 minutes	dose may be repeated depending on intracranial edema, in consultation with DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excess salivation

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5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other LRS drip
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	continuous during procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	LRS
6.	Volume of injections or administrations (where applicable)	10ml/kg/hr, unless otherwise directed by DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

Other premedications not already listed above

1.	Agent Name*	Dexamethasone
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.05-2 mg/kg on the day before surgery; 0.05-2 mg/kg on the day of surgery, and then tapered over 3-7 days according to animal health in consultation with DCM veterinarian
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other Mannitol
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	over 20-60 minutes

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4.	Frequency of injections or administrations (if using inhalation agent)	dose may be repeated depending on intracranial edema, in consultation with DCM veterinarian
5.	Dose of injections or administrations	0.5-2.2 g/kg
6.	Volume of injections or administrations (where applicable)	typically less than 100 mL

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Antibiotics or Anti-Microbials

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Cefazolin	IM or IV	20-30 mg/kg	works for 8-12 h	prior to surgery then twice a day for 1-5 days
Amikacin	Topical (topical)	0.1-0.3 mL, 250 mg/ml	small volume left in recording chamber at end of procedure	once during procedure. Repeated during chamber cleanings.
Gentamicin	Topical (topical)	0.1-0.3 mL, 50 mg/ml	small volume left in recording chamber at end of procedure	once during procedure. Repeated during chamber cleanings.

Antibiotics or Anti-Microbials

1.	Agent Name*	Cefazolin
2.	Route of Administration*	Other IM or IV
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 h
4.	Frequency of injections or administrations (if using inhalation agent)	prior to surgery then twice a day for 1-5 days
5.	Dose of injections or administrations	20-30 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls for IM

Antibiotics or Anti-Microbials

1.	Agent Name*	Other Amikacin
2.	Route of Administration*	Topical (topical)
3.	Duration of injections or administrations (if using inhalation agent)	small volume left in recording chamber at end of procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once during procedure. Repeated during chamber cleanings.
5.	Dose of injections or administrations	0.1-0.3 mL, 250 mg/ml
6.	Volume of injections or administrations (where applicable)	0.1-0.3 mL

Antibiotics or Anti-Microbials

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1.	Agent Name*	Gentamicin
2.	Route of Administration*	Topical (topical)
3.	Duration of injections or administrations (if using inhalation agent)	small volume left in recording chamber at end of procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once during procedure. Repeated during chamber cleanings.
5.	Dose of injections or administrations	0.1-0.3 mL, 50 mg/ml
6.	Volume of injections or administrations (where applicable)	0.1-0.3 mL

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after	No additional monitoring after the first 4 days.

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	procedure/surgery.	
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	Animals will be monitored closely during initial recovery from anesthesia. After extubation, animals will be returned to their home cage where they will be continuously monitored until they are able to sit up on their own. Once they are sitting they will be monitored every 15-30 minutes for the hour after they are sitting up on their own. For the ensuing four hours, animals will continue to be examined regarding respiration rate, visible signs of activity and alertness, appetite, bleeding, swelling, overall appearance, balance, use of limbs, spontaneous head and eye movements, and signs of pain or discomfort at hourly intervals, and monitored at least twice a day by lab and/or DCM for the following 2 days. Responsiveness to visual and auditory stimulation will be monitored at frequent intervals during the first 2 days. Monitoring will continue at least once per day during day 3 and day 4, thus at least until 96 hours after surgery.

* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Surgery

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2.	Brief Description:*	Pial peel
3.	Species: *	Non-Human Primate (various)
4.	USDA Pain/Distress Category:*	D

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* * * Surgery Info * * *

Surgery Information

1. **Surgery Type:** S-Survival
2. **Surgery Classification** Major

NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

Over time, tissue builds up beneath the artificial dura, making it impossible to visualize the underlying tissue, deliver light for optogenetic activation / inactivation, record light reflected by the brain surface during optical imaging, or difficult to pass an electrode through the artificial dura in order to make neural recordings. It is therefore necessary to periodically remove this tissue.

Prior to surgery, the animal is deprived of food (but not water) for 8-12 hours. Prior to surgery, prophylactic antibiotics are given (Cefazolin 20-30 mg/kg IM BID or equivalent or/and as recommended by DCM veterinary staff; timing of first dose as recommended by DCM veterinary staff). Administration of the antiemetic Metoclopramide (Reglan) @ 0.2-0.3 mg/kg IM SID-BID will be used for animals that show a tendency to get nauseous or vomit either during or following the administration of anesthetic agents. Reglan will be given 10-20 minutes before preanesthetic injections. If nausea continues after recovery from anesthesia it can be given SID-TID as recommended by DCM veterinary staff. In addition, the DCM veterinary staff typically administers an analgesic(s) prior to beginning surgical procedures on the day of surgery. Examples of preoperative analgesics include Buprenorphine, Banamine, etc. Pre-emptive Buprenorphine dose is 0.01 mg/kg IM and @ 0.02-0.03 mg/kg IM every 8-10 hours postoperatively prn as determined by the DCM veterinary staff, or Buprenorphine SR 0.2 mg/kg SC will be administered prior to beginning the surgical procedure. To prevent brain edema we will typically administer Dexamethasone and mannitol. A dose of 0.05-2 mg/kg of Dexamethasone may be given the night before, a dose of 0.05-2 mg/kg the day of surgery and then reduced over time according to animal health in consultation with DCM veterinarians. Mannitol will be administed on the day of surgery, prior to opening the dura mater (0.5-2.2 g/kg IV, over 20-60 minutes).
The use of mannitol as described here is specific to prevent brain edema in procedures that require installation of an artificial dura over the cortex. In these procedures, it is especially important to avoid any damage to the cortical surface, and therefore to avoid any swelling of the brain. Otherwise the cortical surface may become damaged during the durotomy, or during the installation of brim of the artificial dura underneath the dural edge, which would prevent obtaining electrophysiological or optical data from this part of the cortex. It is thus the standard approach of laboratories that use this procedure to administer mannitol at least 30 minutes prior to opening the dura, because mannitol takes time to start working. This approach is used in all labs doing such procedures in macaque monkeys as far as we know, including the [redacted] at the [redacted] the [redacted] the [redacted] the [redacted] with excellent outcomes. This approach has been published in Shtoyerman et al., J Neurosci 2000 doi 10.1523/JNEUROSCI.20-21-08111.2000 [redacted].

The animal is initially anesthetized with ketamine plus glycopyrrolate to reduce salivation. The animal is transferred to the surgical prep area, weighed, and the surgical site is shaved and cleaned, followed by at least 3 scrubs with alcohol, betadine or Nolvasan. Eye lubricant will be applied. IV fluids (LRS) will be administered at a rate of 10ml/kg/hr, unless otherwise directed by the veterinarian). Surgical anesthesia is produced using isoflurane. The animal is intubated, connected to a respirator (typical initial isoflurane concentration: 4%) and the expired gas monitored by a CO2 meter. At this time, the animal is transferred to the surgery room. Once in the surgery room, the animal is mounted in a stereotaxic frame over a heating pad, and EKG electrodes, a rectal temperature probe and an oxygen saturation transducer (percentage of hemoglobin existing as oxyhemoglobin) are connected. Depth of anesthesia is monitored using heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex). To prevent brain edema, hyperventilation may be induced during the surgery, and monitored by expired CO2, in consultation with the DCM veterinarian. Supplemental heat will be provided such as Bair hugger or water recirculating blanket.

Once the animal is stable, the surgeon and (optional) assistant or trainee scrub and put on sterile gowns while the anesthetist continues to monitor the animal. Prior to starting surgery, a sterile drape will cover the animal and surgical table for instruments. Prior to the beginning of surgery all instruments, including implants, will be sterilized (heat, gas, liquid such as cetylcide for 20 minutes and then rinsed with sterile saline/water).

This procedure involves removal of the artificial dura, and use of sterile instruments (such as forceps, scalpel, dural hook, suture, and suction) to gently remove the tissue. The procedure is complete when the pial surface is exposed. At the end, a new artificial dura will be inserted, and a piece of sterile gauze with Amikacin or Gentamycin left in place before closing the chamber (as described in section Durotomy').
If bleeding occurs during the procedure, sterile gelfoam or similar products (with or without thrombin), and/or electrocautery may be used to stop bleeding. Gelfoam we use is freshly taken from a new sterile pack and we apply it under aseptic conditions so the risk of infection is low. Further, gelfoam is an absorbable material and therefore may be left under the skin, as long as an excessive amount is not used.

The animal recovers under observation until responsive and mobile and then is returned to its home cage. Prophylactic antibiotics (previously described) are continued for 3 days or as recommended by in–house veterinarian. Tylenol (10 mg/kg, PO, prn up to qid) or Buprenorphine SR (0.2mg/kg, SC once that lasts for up to 72 hours) or Buprenorphine (0.01-0.03 mg/kg, IM, bid/prn) is given as necessary over the next 2 days. Cefazolin is given IM post-operatively as suggested by DCM staff.

Potential reasons for multiple surgeries.
This procedure will sometimes need to be repeated, typically only several months later, if new tissue buildup prevents recordings and more data needs to be collected from the same chamber.

We will follow the IACUC Policy: Anesthesia, Surgery and Post Procedural Care for Non-Rodent Mammals for all survival surgical

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procedures.

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

Potential complications include infection along wound margins or under the artificial dura. We take strong precautions to minimize the risk of infection and to eliminate or contain any infection if it does occur. Using the procedure described above, the (b) (4) at the (b) (4) (b) (6), (b) (7)(C) has not seen this procedure to be complicated by infection. If infection should occur, this may require the elective removal of the implant. Systemic and/or local antibiotic treatment will be started in consultation with the DCM veterinarian. Another possible complication is postoperative bleeding under the dura/artificial dura. We minimize the risk by proper hemostasis during the surgery as described above. Often a small amount of blood is observed under the artificial dura during the first chamber cleaning, which doesn't require any treatment and disappears within a few weeks. Should there be a substantial amount of blood that exerts pressure on the underlying tissue, we will start appropriate treatment in consultation with the DCM veterinarian. This may include elective replacement of the artificial dura so that the clot can be removed.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

As described in section Routine Maintenance of Recording Chamber With Artificial Dura', recording chambers with artificial dura are cleaned at least every 5 days. During cleanings routine inspection of the wound margins and the chamber occurs, as well as of the tissue underneath the transparent artificial dura.

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

As described in the Flowchart Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Typically 5-7 hours of the animal under anesthesia.

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* * * Personnel Details * * *

Personnel Details

Personnel Details

Select Names of personnel who will be performing this surgical procedure for this protocol. * Check all that apply.
(b) (6), (b) (7)(C)

Personnel Details

1. Select Names of personnel who will be performing this surgical procedure for this protocol.* Check all that apply. (b) (6), (b) (7)(C)

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

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Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg		once to prevent excess salivation
LRS drip	Intravenous (IV)	LRS	continuous during procedure	once
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID
Dexamethasone	Intramuscularly (IM)	0.05-2 mg/kg on the day before surgery; 0.05-2 mg/kg on the day of surgery, and then tapered over 3-7 days according to animal health in consultation with DCM veterinarian		SID-BID
Mannitol	Intravenous (IV)	0.5-2.2 g/kg	over 20-60 minutes	dose may be repeated depending on intracranial swelling, in consultation with DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excess salivation

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5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other LRS drip
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	continuous during procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	LRS
6.	Volume of injections or administrations (where applicable)	10ml/kg/hr, unless otherwise directed by DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

Other premedications not already listed above

1.	Agent Name*	Dexamethasone
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.05-2 mg/kg on the day before surgery; 0.05-2 mg/kg on the day of surgery, and then tapered over 3-7 days according to animal health in consultation with DCM veterinarian
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other Mannitol
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	over 20-60 minutes

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4.	Frequency of injections or administrations (if using inhalation agent)	dose may be repeated depending on intracranial swelling, in consultation with DCM veterinarian
5.	Dose of injections or administrations	0.5-2.2 g/kg
6.	Volume of injections or administrations (where applicable)	typically less than 100 mL

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Antibiotics or Anti-Microbials

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Cefazolin	IM or IV	20-30 mg/kg	works for 8-12 h	prior to surgery then twice a day for 1-5 days
Amikacin	Topical (topical)	0.1-0.3 mL, 250 mg/ml	small volume left in recording chamber at end of procedure	once during procedure. Repeated during chamber cleanings.
Gentamicin	Topical (topical)	0.1-0.3 mL, 50 mg/ml	small volume left in recording chamber at end of procedure	once during procedure. Repeated during chamber cleanings.

Antibiotics or Anti-Microbials

1.	Agent Name*	Cefazolin
2.	Route of Administration*	Other IM or IV
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 h
4.	Frequency of injections or administrations (if using inhalation agent)	prior to surgery then twice a day for 1-5 days
5.	Dose of injections or administrations	20-30 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls for IM

Antibiotics or Anti-Microbials

1.	Agent Name*	Other Amikacin
2.	Route of Administration*	Topical (topical)
3.	Duration of injections or administrations (if using inhalation agent)	small volume left in recording chamber at end of procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once during procedure. Repeated during chamber cleanings.
5.	Dose of injections or administrations	0.1-0.3 mL, 250 mg/ml
6.	Volume of injections or administrations (where applicable)	0.1-0.3 mL

Antibiotics or Anti-Microbials

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1.	Agent Name*	Gentamicin
2.	Route of Administration*	Topical (topical)
3.	Duration of injections or administrations (if using inhalation agent)	small volume left in recording chamber at end of procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once during procedure. Repeated during chamber cleanings.
5.	Dose of injections or administrations	0.1-0.3 mL, 50 mg/ml
6.	Volume of injections or administrations (where applicable)	0.1-0.3 mL

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after	No additional monitoring after the first 4 days.

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	procedure/surgery.	
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	Animals will be monitored closely during initial recovery from anesthesia. After extubation, animals will be returned to their home cage where they will be continuously monitored until they are able to sit up on their own. Once they are sitting they will be monitored every 15-30 minutes for the hour after they are sitting up on their own. For the ensuing four hours, animals will continue to be examined regarding respiration rate, visible signs of activity and alertness, appetite, bleeding, swelling, overall appearance, balance, use of limbs, spontaneous head and eye movements, and signs of pain or discomfort at hourly intervals, and monitored at least twice a day by lab and/or DCM for the following 2 days. Responsiveness to visual and auditory stimulation will be monitored at frequent intervals during the first 2 days. Monitoring will continue at least once per day during day 3 and day 4, thus at least until 96 hours after surgery.

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Non-Surgical Procedure Under Anesthesia

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2.	**Brief Description:***	Dura thinning
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	D

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

Once the craniotomy is opened, the tissue starts growing above the dura. Over time, this tissue thickens and calcifies, allowing only 4–6 weeks for transdural recording. Later recordings can be made with guide tubes, but some surface cortical structures can only be accessed with transdural electrodes. Under sterile conditions, we will occasionally anesthetize the animal in the [redacted] Prior to anesthesia, the animal will be fasted for between 8-12 hours. While the animal is under general inhalational anesthesia (typically combination of N2O/Isoflurane) and in sternal recumbency with or without a stereotaxic device and to remove a layer of tissue above the dura.

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

None expected but may be caused if the dura is scratched or punctured.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Anesthetized animals will be continuously monitored until they have recovered from anesthesia.

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

Veterinary staff will be consulted if the dura is injured.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Up to 1 hour.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation

Other premedications not already listed above

1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation

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5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after procedure/surgery.	N/A
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	N/A

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Behavioral or Physiological Testing
2.	Brief Description:*	Optical imaging
3.	Species: *	Non-Human Primate (various)
4.	USDA Pain/Distress Category:*	C

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

In this procedure, the animal will be positioned comfortably in the primate chair, transported to the lab and head fixed. A microscope is used to image optical signals from the cortical tissue in the recording chamber (either widefield imaging or multiphoton imaging). Before the imaging session, the chamber will be cleaned with sterile saline as described in the chamber cleaning procedure. The microscope can rotate such that the animal is comfortably seated in normal upright position during the recording session. For these

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experiments, recording chambers with an artificial dura are used to have optical access to the brain. During imaging, it is necessary to minimize brain movements due to pulsations from heartbeat and breathing. Therefore, after irrigating the chamber, we will position a sterile insert (consisting of a metal ring connected to a glued stack of coverslips) on top of the artificial dura, to provide gentle downward pressure during imaging on the insert, similar as in our electrophysiological experiments. Then we will install a sterile metal imaging platform on top of the chamber, which can be connected to the microscope, to further improve stability. This part will be manipulated using sterile gloves and mounted on the chamber under sterile conditions, after the chamber has been cleaned. Then the microscope head with objective lens, or the camera lens (for widefield imaging) will be positioned over the chamber. The microscope uses either an air-immersion lens. We will then surround the chamber and microscope with light-attenuating material, to allow for optical imaging. After imaging, the platform and insert are removed, and the chamber will be irrigated with sterile saline again. As in our standard artificial dura chamber, we place a small piece of sterile gauze soaked with a small volume of topical antibiotic in the chamber before closing the chamber, and use a silicone gasket between the chamber and the cap to properly seal the chamber and further reduce risk of infection.

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

If the monkey shows overt signs of discomfort (e.g., grimacing, vocalization, squirming, etc.) then the imaging session will be discontinued.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Optical imaging sessions do not involve penetration of tissue with foreign objects. During the session behavioral assays (pupils, trial initiation time, and other measures of motivation) will be used, along with clinical observation on CCTV to determine if any problems arise.

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

As described in the FlowChart, Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Up to 8 hours, including cleaning of the chamber.

* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

Basic Covered Species Monitoring

Body Temperature, Respiratory Rate, etc.

Other with Specialized Equipment (Describe below)

Procedures

1.	**Procedure Type:***	Surgery
2.	**Brief Description:***	Viral injections without a recording chamber
3.	**Species: ***	Non-Human Primate (various)
4.	**USDA Pain/Distress Category:***	D

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* * * Surgery Info * * *

Surgery Information

1. **Surgery Type:** S-Survival
2. **Surgery Classification** Major

NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.

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* * * Procedure Description * * *

Procedure Description

1. **Detailed Procedure Description**

The purpose of viral injections in the brain is to express certain proteins in targeted locations. Such proteins can be for example opsins for optogenetics studies, GCaMP for optical imaging of calcium signals, or fluorescent proteins for histology. This procedure concerns injections in a region that does not have a recording chamber (e.g. for histology, or in case virus is injected prior to chamber implantation).

Prior to surgery, the animal is deprived of food (but not water) for at least 8-12 hours. Prior to surgery, prophylactic antibiotics are given (Cefazolin 20-30 mg/kg IM BID or equivalent or/and as recommended by DCM veterinary staff; timing of first dose as recommended by DCM veterinary staff). Administration of the antiemetic Metoclopramide (Reglan) @ 0.2-0.3 mg/kg IM SID-BID will be used for animals that show a tendency to get nauseous or vomit either during or following the administration of anesthetic agents. Reglan will be given 10-20 minutes before preanesthetic injections. If nausea continues after recovery from anesthesia it can be given SID-TID as recommended by DCM veterinary staff. In addition, the DCM veterinary staff typically administers an analgesic(s) prior to beginning surgical procedures on the day of surgery. Examples of preoperative analgesics include Buprenorphine, Banamine, etc. Pre-emptive Buprenorphine dose is 0.01 mg/kg IM and @ 0.02-0.03 mg/kg IM every 8-10 hours postoperatively prn as determined by the DCM veterinary staff, or Buprenorphine SR 0.2 mg/kg SC will be administered prior to beginning the surgical procedure. Local infusion of bupivicaine/lidocaine will be applied at the incision sites pre-op to help reduce pain/distress during recovery. To prevent brain edema we will typically administer Dexamethasone and mannitol. A dose of 0.05-2 mg/kg of Dexamethasone may be given the night before, a dose of 0.05-2 mg/kg the day of surgery and then reduced over time according to animal health in consultation with DCM veterinarians. Mannitol will be administered on the day of surgery, prior to opening the dura mater (0.5-2.2 g/kg IV, over 20-60 minutes).
The use of mannitol as described here is specific to prevent brain edema in procedures that require installation of an artificial dura over the cortex. In these procedures, it is especially important to avoid any damage to the cortical surface, and therefore to avoid any swelling of the brain. Otherwise the cortical surface may become damaged during the durotomy, or during the installation of brim of the artificial dura underneath the dural edge, which would prevent obtaining electrophysiological or optical data from this part of the cortex. It is thus the standard approach of laboratories that use this procedure to administer mannitol at least 30 minutes prior to opening the dura, because mannitol takes time to start working. This approach is used in all labs doing such procedures in macaque monkeys as far as we know, including the (b) (4) at the (b) (4) (b) (7)(C), (b) (6) , the (b) (4) (b) (4) , the (b) (4) the (b) (4) , with excellent outcomes. This approach has been published in Shtoyerman et al., J Neurosci 2000 doi 10.1523/JNEUROSCI.20-21-08111.2000 (b) (4)

The animal is initially anesthetized with ketamine plus glycopyrrolate to reduce salivation. The animal is transferred to the surgical prep area, weighed, and the surgical site is shaved and cleaned, followed by at least 3 scrubs with alcohol, betadine or Nolvasan. Eye lubricant will be applied. IV fluids (LRS) will be administered at a rate of 10ml/kg/hr, unless otherwise directed by the veterinarian). Surgical anesthesia is produced using isoflurane. The animal is intubated, connected to a respirator (typical initial isoflurane concentration: 4%) and the expired gas monitored by a CO2 meter. At this time, the animal is transferred to the surgery room. Once in the surgery room, the animal is mounted in a stereotaxic frame over a heating pad, and EKG electrodes, a rectal temperature probe and an oxygen saturation transducer (percentage of hemoglobin existing as oxyhemoglobin) are connected. Depth of anesthesia is monitored using heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex). To prevent brain edema, hyperventilation may be induced during the surgery, and monitored by expired CO2, in consultation with the DCM veterinarian. Supplemental heat will be provided such as Bair hugger or water recirculating blanket. Once the animal is stable, the surgeon and (optional) assistant or trainee scrub and put on sterile gowns while the anesthetist continues to monitor the animal. Prior to starting surgery, a sterile drape will cover the animal and surgical table for instruments. Prior to the beginning of surgery all instruments, including implants, will be sterilized (heat, gas, liquid such as cetylcide for 20 minutes and then rinsed with sterile saline/water).

One or more craniotomies and durotomies will be performed prior to the injections in the same procedure. A scalp incision, typically 2-3 inches to give us sufficient view over the skull area where the craniotomy(s) will be placed, is made using instruments such as sterile scalpel blade for the incision, scissors for blunt dissection and incision and forceps to hold the tissue. A periosteal elevator may be used to remove any soft tissue and the periosteum from the section of the bone where the craniotomy will be made/ chamber placed. A craniotomy or craniotomies will be made in the skull to expose the underlying dura mater. The number of craniotomies made will depend on the number of brain areas that need to be injected. In order to reduce the number of animals used in our experiments, we may, as needed, make more than one craniotomy, to access the target areas. We will ensure the integrity of the calvarium by ensuring that there is a region of bone at least 3 mm between two adjacent craniotomies, and by properly closing the calvarium at the end of the procedure to return to integrity (see below). The number of craniotomies is limited, to some extent by viruses to be injected. For example, in testing the efficacy of viruses that are taken up by synaptic terminals, we would typically only make one craniotomy per hemisphere, because multiple craniotomies in one hemisphere would lead to protein expression that could be derived from any of the craniotomies in a single hemisphere. The size of each craniotomy will depend on the number and locations of injections to be made, and the requirement that we have good access to the dura mater, so that we can open it safely, and make the injections. The maximum anticipated craniotomy size is 22 mm in diameter; this is the size of craniotomies made for artificial dura chambers in our lab. The dura will be cut and a glass micropipette or Hamilton syringe filled with virus lowered into the brain. Micropipette or syringe are appropriately sterilize before use using either UV irradiation, 10% bleach or cetylcide followed by rinse with sterile saline. The viruses to be injected include the following recombinant, replication incompetent viruses: AAV, lentivirus. The virus will be injected using 1) a calibrated sterile glass micropipette coupled to tubing and a syringe, with which pressure can be controlled manually during the injection (monitored with a manometer), or 2), a Hamilton syringe with a sterile pipette tip. Preventing

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damage of the tissue is of primary importance to us, and is in fact why we use these methods to deliver virus, when feasible, instead of an injectrode. Pressure and volume of injected virus and depth of pipette are precisely controlled. The injection speed is 50-100 nanoliter/min. When selecting locations for the injections, we avoid key structures such as blood vessels. We carefully monitor the surface of the brain for swelling. Most importantly, histological analysis of brain tissue after injection of virus has shown very minimal damage to brain tissue, and clear protein expression, indicating healthy neurons at the injection site. The pipette will then be removed.

At the end of the procedure we will usually cover the exposed pial surface with a thin sterile sheet (made of polyurethane Tecoflex, silicone, Polydimethylsiloxane (General Electric), or a similar material). This is thus an artificial dura, but of a different shape than the one used in the chamber (no upstanding part). The native dura flap that was retracted following durotomy is then folded over this sheet, and may be secured to it with a small amount of Vetbond. An alternative approach, in cases in which the opening in the dura is small, is to cover this opening with gelfoam, to promote sealing and prevent leakage of cerebrospinal fluid. If feasible, the bone flap that was removed to create the craniotomy (or alternatively an artificial bone flap made of acrylic or bone cement) will be placed over the dura. Gel foam may be used to fill any gap that exists between the bone fragment and the surrounding bone. We will add sterile bone wax over the gap or use vetbond adhesive to close the seam between the bone fragment and the surrounding bone, as is often done in the (b) (4) at the (b) (4), (b) (6), (b) (7)(C) Then the skin will be sutured closed.

If bleeding occurs during the procedure, sterile gelfoam or similar products (with or without thrombin to further control bleeding), or bipolar electrocautery may be used to stop bleeding. Gelfoam we use is freshly taken from a new sterile pack and we apply it under aseptic conditions so the risk of infection is low. Further, gelfoam is an absorbable material and therefore may be left under the skin, as long as an excessive amount is not used.
We will follow the IACUC Policy: Anesthesia, Surgery and Post Procedural Care for Non-Rodent Mammals for all survival surgical procedures.
The animal recovers under observation until responsive and mobile and then is returned to its home cage. Prophylactic antibiotics (previously described) are continued for 3 days or as recommended by in-house veterinarian. Tylenol (10 mg/kg, PO, prn up to qid) or Buprenorphine SR (0.2mg/kg, SC once that lasts for up to 72 hours) or Buprenorphine (0.01-0.03 mg/kg, IM, bid/prn) is given as necessary over the next 2 days. Cefazolin is given IM post-operatively as suggested by DCM staff.

Potential reasons for multiple surgeries.
There are experimental justifications for injections to be made at different times or repeated. If the length of time needed exceeds reasonable time for anesthetized animals, we may repeat the injection procedure to target additional sites in a separate session. Two other cases in which we may repeat the craniotomy and viral injection procedure are: 1. when we need to compare protein expression following injection of two candidate viral constructs. The scientific justification for this is that testing one virus in each of two separate animals would introduce a confound in which differences in protein expression patterns could be attributable to either differences in the virus or differences in protein expression across animals. To enable us to determine differences in protein expression across viruses, the injections need to be made in the same animal. Given the time required for injection of one virus, it may not be feasible to inject additional viruses in one surgical session. A typical injection time for a single site is 5-15 minutes. We will try to keep the duration the animal is under anesthesia under seven hours though we may extend the surgery with veterinary approval, depending on animal health. The duration of the surgery will govern the number of injections made within that surgery. It is not feasible to reopen the original craniotomy to make the second injection, as this would introduce another difference: that the second injection would be made in tissue that had previously been exposed in the first surgery. Thus, a second surgery is required to create a craniotomy within which to test the second virus. 2. when we need to test protein expression patterns over two points in time. In this case, we inject the virus in one craniotomy, and then wait a period of time comparable to the time we anticipate using the virus in optogenetic activation experiments (in other animals) before opening a second craniotomy to inject the virus. After time has passed to allow protein expression in the second craniotomy, we will euthanize the animal and perform histology to examine protein expression patterns at the two locations, corresponding to two different periods of protein expression.

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

Potential complications include bacterial infection. We take strong precautions to minimize the risk of infection and to eliminate or contain any bacterial infection if it does occur. Systemic and/or local antibiotic treatment will be started in consultation with the DCM veterinarian.

3. **Describe post procedure monitoring, observation schedules, and treatment that will be performed.**

Standard post procedure monitoring of wound margins and clinical status of the animal will allow us to detect complications promptly, and start the appropriate treatment in consultation with the DCM veterinarian.

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

As described in the Flowchart Sequence and Timing section.

5. **What is the duration of the procedure, from anesthesia to wake up?**

Typically 5-7 hours of the animal under anesthesia.

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* * * Personnel Details * * *

Personnel Details

Personnel Details

Select Names of personnel who will be performing this surgical procedure for this protocol. * Check all that apply.
[redacted]

Personnel Details

1. Select Names of personnel who will be performing this surgical [redacted] procedure for this protocol.*
Check all that apply.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

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Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg		once to prevent excess salivation
LRS drip	Intravenous (IV)	LRS	continuous during procedure	once
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID
Dexamethasone	Intramuscularly (IM)	0.05-2 mg/kg on the day before surgery; 0.05-2 mg/kg on the day of surgery, and then tapered over 3-7 days according to animal health in consultation with DCM veterinarian		SID-BID
Mannitol	Intravenous (IV)	0.5-2.2 g/kg	over 20-60 minutes	dose may be repeated depending on intracranial swelling, in consultation with DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excess salivation

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5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other LRS drip
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	continuous during procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	LRS
6.	Volume of injections or administrations (where applicable)	10ml/kg/hr, unless otherwise directed by DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

Other premedications not already listed above

1.	Agent Name*	Dexamethasone
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.05-2 mg/kg on the day before surgery; 0.05-2 mg/kg on the day of surgery, and then tapered over 3-7 days according to animal health in consultation with DCM veterinarian
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other Mannitol
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	over 20-60 minutes

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4.	Frequency of injections or administrations (if using inhalation agent)	dose may be repeated depending on intracranial swelling, in consultation with DCM veterinarian
5.	Dose of injections or administrations	0.5-2.2 g/kg
6.	Volume of injections or administrations (where applicable)	typically less than 100 mL

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Antibiotics or Anti-Microbials

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Cefazolin	IM or IV	20-30 mg/kg	works for 8-12 h	prior to surgery then twice a day for 1-5 days

Antibiotics or Anti-Microbials

1.	Agent Name*	Cefazolin
2.	Route of Administration*	Other IM or IV
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 h
4.	Frequency of injections or administrations (if using inhalation agent)	prior to surgery then twice a day for 1-5 days
5.	Dose of injections or administrations	20-30 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls for IM

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours

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4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (usually 4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after procedure/surgery.	No additional monitoring after the first 4 days.
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	Animals will be monitored closely during initial recovery from anesthesia. After extubation, animals will be returned to their home cage where they will be continuously monitored until they are able to sit up on their own. Once they are sitting they will be monitored every 15-30 minutes for the hour after they are sitting up on their own. For the ensuing four hours, animals will continue to be examined regarding respiration rate, visible signs of activity and alertness, appetite, bleeding, swelling, overall appearance, balance, use of limbs, spontaneous head and eye movements, and signs of pain or discomfort at hourly intervals, and monitored at least twice a day by lab and/or DCM for the following 2 days. Responsiveness to visual and auditory stimulation will be monitored at frequent intervals during the first 2 days. Monitoring will continue at least once per day during day 3 and day 4, thus at least until 96 hours after surgery.

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

1.	Procedure Type:*	Surgery
2.	Brief Description:*	Recording chamber removal
3.	Species: *	Non-Human Primate (various)
4.	USDA Pain/Distress Category:*	D

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* * * Surgery Info * * *

Surgery Information

1. Surgery Type: S-Survival
2. Surgery Classification Major

NOTE: A major operative procedure is any surgical intervention that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.

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* * * Procedure Description * * *

Procedure Description

1. Detailed Procedure Description

Removal of a recording chamber would be done when no more physiological data needs to be recorded from the brain tissue in the recording chamber. The removal contributes to the animal's comfort (reduces the number of cleaning sessions). Another, rare, reason for removal is that the implant is no longer stable and repair is not possible.

Prior to surgery, the animal is deprived of food (but not water) for 8-12 hours. Prior to surgery, prophylactic antibiotics are given (Cefazolin 20-30 mg/kg IM BID or equivalent or/and as recommended by DCM veterinary staff; timing of first dose as recommended by DCM veterinary staff). Administration of the antiemetic Metoclopramide (Reglan) @ 0.2-0.3 mg/kg IM SID-BID will be used for animals that show a tendency to get nauseous or vomit either during or following the administration of anesthetic agents. Reglan will be given 10-20 minutes before preanesthetic injections. If nausea continues after recovery from anesthesia it can be given SID-TID as recommended by DCM veterinary staff. In addition, the DCM veterinary staff typically administers an analgesic(s) prior to beginning surgical procedures on the day of surgery. Examples of preoperative analgesics include Buprenorphine, Banamine, etc. Pre-emptive Buprenorphine dose is 0.01 mg/kg IM and @ 0.02-0.03 mg/kg IM every 8-10 hours postoperatively prn as determined by the DCM veterinary staff, or Buprenorphine SR 0.2 mg/kg SC will be administered prior to beginning the surgical procedure.

The animal is initially anesthetized with ketamine plus glycopyrrolate to reduce salivation. The animal is transferred to the surgical prep area, weighed, and the surgical site is shaved and cleaned, followed by at least 3 scrubs with alcohol, betadine or Nolvasan. Eye lubricant will be applied. IV fluids (LRS) will be administered at a rate of 10ml/kg/hr, unless otherwise directed by the veterinarian). Surgical anesthesia is produced using isoflurane. The animal is intubated, connected to a respirator (typical initial isoflurane concentration: 4%) and the expired gas monitored by a CO2 meter. At this time, the animal is transferred to the surgery room. Once in the surgery room, the animal is mounted in a stereotaxic frame over a heating pad, and EKG electrodes, a rectal temperature probe and an oxygen saturation transducer (percentage of hemoglobin existing as oxyhemoglobin) are connected. If an incision will be made (which is sometimes necessary to make sure that the soft tissues/skin can properly cover the area of the chamber), a sterile marker will be used to draw a line of where the incision line will be made and Lidocaine 1-2mg/kg mixed with Bupivicaine 1-2mg/kg will be injected ID along this line for local analgesia. Depth of anesthesia is monitored using heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (4–5%), and reflexes (e.g., toe pinch, corneal reflex). Supplemental heat will be provided such as Bair hugger or water recirculating blanket.

Once the animal is stable, the surgeon and (optional) assistant or trainee scrub and put on sterile gowns while the anesthetist continues to monitor the animal. Prior to starting surgery, a sterile drape will cover the animal and surgical table for instruments. Prior to the beginning of surgery all instruments will be sterilized (heat, gas, liquid such as cetylcide for 20 minutes and then rinsed with sterile saline/water).

The cranial implant is surgically removed. Periosteal tissues are repositioned. Sterile gelfoam or similar material may be inserted to cover tissue if needed. A sterile disc made from acrylic or bone cement may be used to replace bone defects if needed. The skin is sutured over the cranium. External sutures to close the skin will be non-absorbable (e.g. Nylon, Polypropylene). Sterile surgical staples are also often used to close the skin.

The animal recovers under observation until responsive and mobile and then is returned to its home cage. Prophylactic antibiotics (previously described) are continued for 3 days or as recommended by in–house veterinarian. Tylenol (10 mg/kg, PO, prn up to qid) or Buprenorphine SR (0.2mg/kg, SC once that lasts for up to 72 hours) or (buprenorphine) (0.01-0.03 mg/kg, IM, bid/prn) is given as necessary over the next 2 days. Cefazolin is given IM post-operatively as suggested by DCM staff.

Non-absorbable sutures or staples will be removed after 7-10 days.

Potential reasons for multiple surgeries.
In case of a removal due to instability of the chamber, after a period sufficient for recovery of cranial tissues (generally no less than 6 months), the implant may be replaced following exactly the same procedure as the original installation.

We will follow the IACUC Policy: Anesthesia, Surgery and Post Procedural Care for Non-Rodent Mammals for all survival surgical procedures.

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

Potential complication involves infections along wound margins. We take strong precautions to minimize the risk of infection and to eliminate or contain any infection if it does occur.

3. Describe post procedure monitoring, observation schedules, and treatment that will be performed.

In addition to the routine inspection and cleaning of skin margins, we apply topical antibiotic ointment if there are signs of infection, and we consult closely with the veterinary staff about appropriate topical and/or systemic treatments to be used.

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

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As described in the Flowchart Sequence and Timing section.

5. What is the duration of the procedure, from anesthesia to wake up?

Typically 5-7 hours of the animal under anesthesia.

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* * * Personnel Details * * *

Personnel Details

Personnel Details

Select Names of personnel who will be performing this surgical procedure for this protocol. * Check all that apply.
[redacted]

Personnel Details

1. Select Names of personnel who will be performing this surgical [redacted] procedure for this protocol.*
Check all that apply.

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* * * Anesthetic Regimen * * *

Anesthetic Regimen

Parameters used to monitor during anesthesia depth:

Standard Rodent Monitoring

Toe/Tail pinch every 15 minutes, Visual Observation of Tissue Color, Heart Rate, Respiratory Rate, etc.

X **Basic Covered Species Monitoring**

Body Temperature, Respiratory Rate, etc.

X **Other with Specialized Equipment (Describe below)**

Optional EKG or indirect blood pressure monitoring may also be performed during anesthesia sessions.

Anesthetic Agents

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Isoflurane	Inhalation (IN)	1-5%	continuous	continuous
Nitrous Oxide	Inhalation (IN)	20-70%	continuous	continuous
Ketamine	Intramuscularly (IM)	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.	works for up to 30 minutes	once, if additional doses are required a lower dose will be used.
Dexmedetomidine	Intramuscularly (IM)	0.01-0.03 mg/kg	works up to 30 minutes	once in combination with low dose ketamine (3-5mg/kg)

Anesthetic Agents

1.	Agent Name*	Isoflurane
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous
4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	1-5%
6.	Volume of injections or administrations (where applicable)	

Anesthetic Agents

1.	Agent Name*	Nitrous Oxide
2.	Route of Administration*	Inhalation (IN)
3.	Duration of injections or administrations (if using inhalation agent)	continuous

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4.	Frequency of injections or administrations (if using inhalation agent)	continuous
5.	Dose of injections or administrations	20-70%
6.	Volume of injections or administrations (where applicable)	N/A

Anesthetic Agents

1.	Agent Name*	Ketamine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once, if additional doses are required a lower dose will be used.
5.	Dose of injections or administrations	10-15 mg/kg if used alone. 3-5 mg/kg if used in combination with Dexmedetomidine.
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Anesthetic Agents

1.	Agent Name*	Dexmedetomidine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 30 minutes
4.	Frequency of injections or administrations (if using inhalation agent)	once in combination with low dose ketamine (3-5mg/kg)
5.	Dose of injections or administrations	0.01-0.03 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL

Other premedications not already listed above

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atropine	Intramuscularly (IM)	0.04-0.05 mg/kg	once per procedure	once to prevent excessive salivation
Glycopyrrolate	Intramuscularly (IM)	13-17 microgram/kg		once to prevent excess salivation
LRS drip	Intravenous (IV)	LRS	continuous during procedure	once
Metoclopramide (reglan)	Intramuscularly (IM)	0.2-0.3 mg/kg		SID-BID

Other premedications not already listed above

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1.	Agent Name*	Atropine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	once per procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excessive salivation
5.	Dose of injections or administrations	0.04-0.05 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Glycopyrrolate
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	once to prevent excess salivation
5.	Dose of injections or administrations	13-17 microgram/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Other premedications not already listed above

1.	Agent Name*	Other LRS drip
2.	Route of Administration*	Intravenous (IV)
3.	Duration of injections or administrations (if using inhalation agent)	continuous during procedure
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	LRS
6.	Volume of injections or administrations (where applicable)	10ml/kg/hr, unless otherwise directed by DCM veterinarian

Other premedications not already listed above

1.	Agent Name*	Other Metoclopramide (reglan)
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	
4.	Frequency of injections or administrations (if using inhalation agent)	SID-BID
5.	Dose of injections or administrations	0.2-0.3 mg/kg
6.	Volume of injections or administrations (where applicable)	

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* * * Perioperative Care * * *

Perioperative Care

Pre-Operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Buprenorphine	Intramuscularly (IM)	0.01-0.02 mg/kg	works for 8-12 hours	twice a day for 2 days
Buprenorphine Sustained Release	Subcutaneous (SC)	0.2 mg/kg	works for 72 hours	once prior to surgery
Flunixin Meglumine	Intramuscularly (IM)	0.5-1.0 mg/kg	works for up to 24 hours	once or twice a day

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 hours
4.	Frequency of injections or administrations (if using inhalation agent)	twice a day for 2 days
5.	Dose of injections or administrations	0.01-0.02 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Buprenorphine Sustained Release
2.	Route of Administration*	Subcutaneous (SC)
3.	Duration of injections or administrations (if using inhalation agent)	works for 72 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once prior to surgery
5.	Dose of injections or administrations	0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Pre-Operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works for up to 24 hours
4.	Frequency of injections or administrations (if using inhalation agent)	once or twice a day

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5.	Dose of injections or administrations	0.5-1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Antibiotics or Anti-Microbials

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Cefazolin	IM or IV	20-30 mg/kg	works for 8-12 h	prior to surgery then twice a day for 1-5 days

Antibiotics or Anti-Microbials

1.	Agent Name*	Cefazolin
2.	Route of Administration*	Other IM or IV
3.	Duration of injections or administrations (if using inhalation agent)	works for 8-12 h
4.	Frequency of injections or administrations (if using inhalation agent)	prior to surgery then twice a day for 1-5 days
5.	Dose of injections or administrations	20-30 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls for IM

Post-operative Monitoring

Note: A minimum of 24 hours of post-operative analgesia must be provided for minor surgical procedures and a minimum of 48 hours of post-operative analgesia must be provided for major operative procedures. All animals must be monitored for 96 hours (4 DAYS) following surgery regardless of when analgesic administration ceased.

Post-operative Analgesics

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Flunixin Meglumine	Intramuscularly (IM)	0.5 - 1.0 mg/kg	works up to 24 hours	

Post-operative Analgesics

1.	Agent Name*	Flunixin Meglumine
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	works up to 24 hours

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4.	Frequency of injections or administrations (if using inhalation agent)	
5.	Dose of injections or administrations	0.5 - 1.0 mg/kg
6.	Volume of injections or administrations (where applicable)	no more than 2 mls

Describe what parameters will be monitored during surgery to ensure proper analgesia.

heart rate (desired range 90–130 for isoflurane), respiration rate (15–20/min), O2 saturation (93–100%), expired CO2 (4–5%), and reflexes (e.g., toe pinch, corneal reflex).

1.	Personnel Responsible for Monitoring Recovery	All lab personnel
2.	Immediate Recovery from anesthesia - What is the duration and frequency of the monitoring?	continuous until the animal recovers from anesthesia
3.	Post-recovery - What is the duration and frequency of the monitoring ? "Post-recovery" is the 3-10 day period after procedure/surgery.	No additional monitoring after the first 4 days.
4.	What signs or parameters in recovery period are monitored? In post-recovery monitoring, what are you looking for?	Animals will be monitored closely during initial recovery from anesthesia. After extubation, animals will be returned to their home cage where they will be continuously monitored until they are able to sit up on their own. Once they are sitting they will be monitored every 15-30 minutes for the hour after they are sitting up on their own. For the ensuing four hours, animals will continue to be examined regarding respiration rate, visible signs of activity and alertness, appetite, bleeding, swelling, overall appearance, balance, use of limbs, spontaneous head and eye movements, and signs of pain or discomfort at hourly intervals, and monitored at least twice a day by lab and/or DCM for the following 2 days. Responsiveness to visual and auditory stimulation will be monitored at frequent intervals during the first 2 days. Monitoring will continue at least once per day during day 3 and day 4, thus at least until 96 hours after surgery.

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* * * Other Drugs Utilized * * *

Other Drugs Utilized

Other Drugs Utilized

Agent Name	Route of Administration	Dose of injections or administrations	Duration of injections or administrations	Frequency of injections or administrations
Atipamezole	Intramuscularly (IM)	0.1-0.2 mg/kg	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine	once

Other Drugs Utilized

1.	Agent Name*	Other Atipamezole
2.	Route of Administration*	Intramuscularly (IM)
3.	Duration of injections or administrations (if using inhalation agent)	may be given after cessation of anesthesia administration, to reverse the dexmedetomidine
4.	Frequency of injections or administrations (if using inhalation agent)	once
5.	Dose of injections or administrations	0.1-0.2 mg/kg
6.	Volume of injections or administrations (where applicable)	less than 2 mL
7.	Purpose, Expected Effect	to reverse the dexmedetomidine

Procedures

Pre-Filled and Custom Procedure Details

Pre-filled Procedures are auto-populated with IACUC-approved descriptions, procedural steps, and details.

Surgical Procedures

1. Will any Animal undergo at least one Surgical Procedure on this protocol? Y

Multiple Survival Surgery & Multiple Major Survival Surgery Justification

2. Will any individual animal undergo more than one (1) Survival Surgery? Y

   If Yes, describe the items below in the text box:

   - Identify the Species and Surgical Procedures

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- **The Time between Procedures**
- **Describe the Criteria used to determine the potential impact on the animal's well-being**
-

To meet the goals and requirements of our work, the number of baseline surgeries in one animal is 7. The first surgery is typically head post implantation (which does not involve opening of a body cavity). Next, we typically perform recording chamber implantation, followed by artificial dura implantation in the installed chamber(s). This is typically a pair of surgeries. Over time, we often repeat this pair of surgeries in the other hemisphere, so that we can compare activity across hemispheres, which necessarily needs to be done in the same animal. After data collection in the chamber in the first hemisphere is completed, this chamber is typically removed in a separate surgery, to reduce infection risk and improve animal comfort by reducing the number of chamber cleanings. Finally, prior to euthanasia there is typically a surgery to inject viral vectors in brain tissue away from the chambers, so that viral expression can be evaluated histologically in the same animal as in which the physiological data was collected. This thus leads to 7 baseline surgeries. In addition to these surgeries, an animal may undergo additional surgeries. It is not possible to precisely predict how many of these will be necessary, because it depends on the experiment as well as on the evolution of the tissue in the chamber. This mirrors the situation in the other NHP labs at WUSM. When needed, such procedures are important to run our research program in a feasible manner, because training a single animal often takes a long time. For example, in an optical imaging experiment on perceptual learning, pial peels are critical in order to restore optical access after granulation tissue grows over the pial surface, so that the evolution of activity of neural responses during the course of learning can be analyzed. Indeed, to make sense of the data, the same neurons need to be imaged over time. Such additional procedures that may be required include:
- pial peel (minor): to regain optical access to the cortical surface if granulation tissue develops on the cortical surface. Such procedures sometimes need to be repeated after a few months if the granulation tissue grows back. In the past we have required this procedure up to 5 times in one animal (both hemispheres combined), but in most animals this is less.
- implant removal: this is often done when data collection in a chamber is no longer necessary, to reduce infection risk and improve animal comfort by removing the need for chamber cleaning. Up to once per chamber.
- modify/repair implant: the most common situation is an external headpost repair, which is a non-surgical procedure under anesthesia (repair top section of the headpost above the skin). Surgical repairs are rare (not required in the majority of animals), but it is critical to be able to do the repair if required, to maintain implant integrity, both for scientific reasons (such that the project can be completed) as for animal welfare.
The time between procedures is described in the flowchart (Sequence and Timing section).
We always consult with DCM veterinary staff when performing any surgical procedures to ensure that the animal is healthy enough to continue with the study. The lab has read and will follow IACUC Policy "Anesthesia, Surgery and Post-Procedural Care for non-rodent mammals".

3. **If animals will participate in multiple surgeries while housed on this protocol, will two (2) or more of those surgical procedures be classified as "Major"? If "Yes", provide additional justification for Multiple Major Survival Surgeries (MMSS).** Y

The goal of NHP neuroscience on visual perception, attention and visually guided behavior is to train NHPs on complex perceptual tasks (taking from 2 months to 1 year to train) and to use these well-trained animals to understand how the brain reconstructs a representation of the external world that allows perceptually guided behavior. This is compatible with the programmatic priority of the NEI at the NIH to understand the systems neurobiology of visual processing, psychophysics, and behavior. To meet our research goals we, and other NHP labs that study complex perception, maintain a small number of highly valuable NHPs (valuable because they are trained to perform behavioral experiments) with multiple craniotomies allowing us to explore the brain with thin electrodes while monkeys willingly participate in our tasks without any discomfort and relatively little risk (e.g. similar electrode recording techniques are used to record from patients). A key point is that a sick or uncomfortable NHP will not be able to be used for research that studies how "normal" healthy animals perceive the world (this is carefully detailed elsewhere in our protocol).
When possible we seek to minimize the total number of surgeries by combining procedures into a single surgery. However, we must balance this against the risks associated with long surgeries. An example is the artificial dura recording chamber. To be able to precisely insert thin multielectrode probes orthogonally relative to the cortical surface while avoiding blood vessels, we replace the native dura with a transparent membrane (artificial dura). In these recording chambers, we often inject viral vectors, so that we can interrogate the cortical circuit using optogenetics (the viral vectors are used to express light-sensitive proteins, opsins, in the targeted tissue). Installing these recording chambers typically occurs in three distinct steps (1 - craniotomy and chamber implantation; 2- durotomy and artificial dura placement; and 3 - viral injection.) Each step is time consuming. If, as we perform a procedure, it becomes clear that conducting the subsequent step in the same surgery would extend the surgery to a duration that is long enough to imperil animal health, we will finish the procedure, and conduct the subsequent procedure in a separate surgery. This judgment will be made in consultation with the DCM veterinarian, and will depend on the state of the animal at the time that the initial procedure is completed, the time taken to perform the initial procedure, and the anticipated duration of the subsequent procedure. So, during a chamber implantation procedure, we will assess whether it is feasible to carry out the durotomy and artificial dura implantation in the same session, within a safe time frame. If the judgment is to carry out the durotomy and artificial dura implantation in a separate surgery, then, at the end of that surgery, we will assess if it is feasible to carry out the viral injections within that surgery or if the viral injection should be carried out in a separate surgery.
Another cause for multiple survival surgeries is linked to the conduct of "unplanned surgeries", in which failed implants are removed, replaced, and/or repaired. Although we take every known precaution to limit implant failure, there is a possibility that implants fail during their lifetime. There are two general strategies one may adopt in the event of failure: (1) removal/replacement/repair, or (2) euthanasia. The former requires, by definition, additional survival surgeries. The latter requires an increase in the total number of experimental animals (the euthanized animal must be replaced) and can result in a substantial and often devastating loss of time devoted to behavioral training and localization and characterization of relevant brain regions. Because such losses are incompatible

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with any degree of effective conduct of this research program, we have elected to conserve experimental animals by (1) removing, replacing, and/or repairing failed implants when possible, and (2) continuing our efforts to reduce implant failure. If implants are removed, the implant(s) may be surgically added again at a later time once the animal/surgical area has healed. In addition, if recording from a chamber is no longer necessary, the chamber is often removed, which contributes to the animal's comfort by reducing the number of required chamber cleanings.

See research.wustl.edu/lamps for system help and training resources.

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* * * Hazards & Other Drugs * * *

Use of Hazardous Agents and Other Drugs in Live Animals

All hazards have been sorted into categories. A link to the whole matrix is available in the Help text if you need assistance identifying the correct picklist selection for your hazard.

Biological Materials, Human / Animal Products, Infectious, and rDNA Agents

1. Are you using Biological Materials, Human/Animal Products, Infectious, or rDNA Agents?* Y

The use of human origin biological material (e.g., hESCs, human tumor cells, human primary tissue, etc.), infectious agents (e.g., lentiviral vectors, pathogenic microbes, etc.), or recombinant or synthetic nucleic acids in animals must be approved by the Institutional Biosafety Committee (IBC).

Biological Materials, Human / Animal Products, Infectious, or rDNA Agents

Specify Material or Agents	Species	Route	Dose	Animal Biosafety Level (ABSL)
Adeno-associated virus, Lentivirus vector	Non-Human Primate (various)	Intracranial (ICa)	up to 2 microliters/site, total injection volume across sites up to 20 microliters	2

Biological Materials, Human / Animal Products, Infectious, or rDNA Agents

1.	Category*	Viral Vector
2.	Specify Material or Agent. If your agent is not listed, check the hazard matrix to verify the correct category or call the IACUC office*	Adeno-associated virus, Lentivirus vector
3.	Species*	Non-Human Primate (various)
4.	Route of Administration*	Intracranial (ICa)
5.	Anatomical Site of administrations* (Enter N/A if this does not apply to your selected route)	brain
6.	Frequency of administrations*	There will be typically at least 2 weeks in between repeated viral injection sessions.
7.	Total number of administrations*	Repeated administrations are often necessary, depending on viral expression, in the same or another recording chamber. Up to 10 viral injection sessions per animal.

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8.	Dose of administrations*	up to 2 microliters/site, total injection volume across sites up to 20 microliters
9.	Volume of administrations*	up to 2 microliters/site, total injection volume across sites up to 20 microliters
10.	Animal Biosafety Level (ABSL) as determined by IBC*	2
11.	Names of personnel who will be using the substance	[redacted]

1a. IBC protocol number(s) for the use of the material or agent in animals. If your protocol is not approved yet, state "pending"*

pending

Toxic Substances, Hazardous Chemicals, and Nanoparticles

2. Are you using Toxic Substances, Hazardous Chemicals or Nanoparticles in animals?* (e.g. carcinogens, chemotherapeutics, reproductive hazards, etc.) Y

Toxic Substances, Hazardous Chemicals, & Nano Particles

Specify Material or Agents	Species	Route	Dose
Gadolinium Chloride	Non-Human Primate (various)	Topical (topical)	0.0015 millimolar
Doxycycline	Non-Human Primate (various)	Oral (PO)	15 mg/kg
Antibiotics (if hazardous and not used for clinical care)	Non-Human Primate (various)	Topical (topical)	amikacin (250 mg/ml,0.1-0.3 mL) or gentamycin (50 mg/ml, 0.1-0.3 mL)
5-Fluorouracil	Non-Human Primate (various)	Topical (topical)	250 mg/10 mL

Toxic Substances, Hazardous Chemicals, & Nano Particles

1.	Category*	Toxin
2.	Specify Material or Agent. If your agent is not listed, check the hazard matrix to verify the correct category or call the IACUC office*	Gadolinium Chloride
3.	Species*	Non-Human Primate (various)
4.	Route of Administration*	Topical (topical)
5.	Anatomical Site of administrations* (Enter N/A if this does not apply to your selected route)	in custom cap of recording chamber
6.	Frequency of administrations*	up to 1x per MRI imaging for chamber location
7.	Total number of administrations*	up to 1x per MRI imaging for chamber location

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8.	Dose of administrations*	0.0015 millimolar
9.	Volume of administrations*	N/A
10.	Names of personnel who will be using the substance	[redacted]

Toxic Substances, Hazardous Chemicals, & Nano Particles

1.	Category*	Reproductive Hazard
2.	Specify Material or Agent. If your agent is not listed, check the hazard matrix to verify the correct category or call the IACUC office*	Doxycycline
3.	Species*	Non-Human Primate (various)
4.	Route of Administration*	Oral (PO)
5.	Anatomical Site of administrations* (Enter N/A if this does not apply to your selected route)	N/A
6.	Frequency of administrations*	intermittent blocks of daily administration (3-7 d)
7.	Total number of administrations*	This drug is used in experiments with tetracycline-controlled transcriptional activation of viral expression. Blocks may be repeated depending on level of viral expression. There will be at least 2 weeks in between blocks of administrations.
8.	Dose of administrations*	15 mg/kg
9.	Volume of administrations*	N/A
10.	Names of personnel who will be using the substance	[redacted]

Toxic Substances, Hazardous Chemicals, & Nano Particles

1.	Category*	Reproductive Hazard
2.	Specify Material or Agent. If your agent is not listed, check the hazard matrix to verify the correct category or call the IACUC office*	Antibiotics (if hazardous and not used for clinical care)
3.	Species*	Non-Human Primate (various)
4.	Route of Administration*	Topical (topical)
5.	Anatomical Site of administrations* (Enter N/A if this does not apply to your selected route)	in recording chamber
6.	Frequency of administrations*	up to once per chamber cleaning
7.	Total number of administrations*	up to once per chamber cleaning
8.	Dose of administrations*	amikacin (250 mg/ml,0.1-0.3 mL) or gentamycin (50 mg/ml, 0.1-0.3 mL)
9.	Volume of administrations*	0.1-0.3 mL
10.	Names of personnel who will be using the substance	[redacted]

Toxic Substances, Hazardous Chemicals, & Nano Particles

1.	Category*	Carcinogen

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2.	Specify Material or Agent. If your agent is not listed, check the hazard matrix to verify the correct category or call the IACUC office*	5-Fluorouracil
3.	Species*	Non-Human Primate (various)
4.	Route of Administration*	Topical (topical)
5.	Anatomical Site of administrations* (Enter N/A if this does not apply to your selected route)	in recording chamber
6.	Frequency of administrations*	2-5x per week
7.	Total number of administrations*	only in the period between craniotomy and durotomy, typically ~2 weeks
8.	Dose of administrations*	250 mg/10 mL
9.	Volume of administrations*	typically <1 mL (enough to cover the dural surface in the chamber)
10.	Names of personnel who will be using the substance	[redacted]

2a. IBC protocol number(s) for the use of the material or agent in animals. If it is not approved yet, please state "pending"*

pending

Radiological Materials and Equipment

The use of radioactive materials and equipment must be permitted through Radiation Safety Office.

3. Are you using Radiological Materials?* N

4. Are you using Radiological Equipment?* Y
(e.g. gamma irradiators, X-ray equipments, CT, fluoroscopy, Class 3B and Class 4 lasers, MRI, NMR, open UV)

Radiological Equipment

Name	Species
Magnets (MRI and NMR)	Non-Human Primate (various)
X-ray producing equipment (CT, microCT, fluoroscopy, IVIS, SPECT)	Non-Human Primate (various)
Lasers (class 3B and class 4)	Non-Human Primate (various)

Radiological Equipment

1.	Name*(contact IACUC office if not listed)	Magnets (MRI and NMR)
2.	Species*	Non-Human Primate (various)
3.	What stipulations from RSC have been included for use of this equipment with animals?	

none

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4. Names of personnel who will be using the material or equipment [redacted]

Radiological Equipment

1. Name*(contact IACUC office if not listed) X-ray producing equipment (CT, microCT, fluoroscopy, IVIS, SPECT)
2. Species* Non-Human Primate (various)
3. What stipulations from RSC have been included for use of this equipment with animals?
   none
4. Names of personnel who will be using the material or equipment [redacted]

Radiological Equipment

1. Name*(contact IACUC office if not listed) Lasers (class 3B and class 4)
2. Species* Non-Human Primate (various)
3. What stipulations from RSC have been included for use of this equipment with animals?
   none
4. Names of personnel who will be using the material or equipment [redacted]

Human Embryonic Stem Cells (hESC) & Human Induced Pluripotent Stem Cells (HiPS)

5. Are you using human pluripotent stem cells, including hESC, hIPSC or their derivatives in animals?* N

The use of human embryonic stem cells (hESC), other human pluripotent stem cells (hPCS) including induced pluripotent stem cells (hiPSC), or their derivatives may require review and approval by the Washington University Embryonic Stem Cell Research Oversight Committee (ESCRO) prior to commencement of the experiments.

Please contact the ESCRO coordinator at 314-747-5571 or reco@wusm.wustl.edu for more information.

You will be expected to provide the ESCRO committee with a summary of the species, procedures to prevent breeding of chimeras, and euthanasia schedule for all animals injected with covered materials.

Non-Hazardous Materials to be Administered to Live Animals

Specify any additional drug or substances administered to live animals used in your experiments that are non-hazardous, and not listed in the questions above.

Do NOT include Anesthesia, Analgesic, or Perioperative care medications that are used and specified in Surgical, Non-Surgical or Euthanasia Procedures.

6. Are you using Other Non-Hazardous Substances to be administered to Live Animals?* Y

Non-Hazardous Materials / Agents to be administered into Live Animals

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Substance Name	Species	Route	Dose
Lidocaine/Prilocaine	Non-Human Primate (various)	Topical (topical)	2.5% lidocaine, 2.5% prilocaine
Lidocaine/Bupivicaine	Non-Human Primate (various)	Intradermal (ID)	1-2 mg/kg Lidocaine mixed with 1-2 mg/kg Bupivicaine

Non-Hazardous Materials / Agents to be administered into Live Animals

1. Substance Name* — Lidocaine/Prilocaine
2. Species* — Non-Human Primate (various)
3. Route of Administration* — Topical (topical)
4. Anatomical Site of administrations* — skin, exact location varies
5. Total number of administrations* — whenever discomfort is noted
6. Dose of administrations* — 2.5% lidocaine, 2.5% prilocaine
7. Volume of administrations* — up to pea-size amount per square centimeter of skin

Non-Hazardous Materials / Agents to be administered into Live Animals

1. Substance Name* — Lidocaine/Bupivicaine
2. Species* — Non-Human Primate (various)
3. Route of Administration* — Intradermal (ID)
4. Anatomical Site of administrations* — prior to surgery, where surgical incision will be made
5. Total number of administrations* — at 1-2 cm intervals along planned surgical incision
6. Dose of administrations* — 1-2 mg/kg Lidocaine mixed with 1-2 mg/kg Bupivicaine
7. Volume of administrations* — 0.1-0.3 mL, at 1-2 cm intervals along planned surgical incision

Non-Pharmaceutical Grade Substances

7. Are you using any Non-pharmaceutical Grade Substances?* Y

7a. Please list the substances and the justification for each in the text box provided.

Typical justifications for use of non-pharmaceutical grade substances.

- Non-phamaceutical grade substance covered by IACUC Policy
- Substance is not available in a pharmaceutical grade formulation
- Previous research completed with non-pharmaceutical grade substance. Use required for continuity of data collection
- Pharmaceutical grade substance has additives or preservatives that could negatively impact data collection

AAV and lentiviral vectors are custom research grade substances that are not available in pharmaceutical grade formulations. Viral vectors used will be produced by viral core labs ([b] (4), [b] (4), [b] (4), [b] (4), [b] (4)). These vectors are provided in physiological buffer (e.g. PBS, OptiPro SFM, HBSS), that have suitable purity and sterility for injection into animals (purification using e.g. Iodaxinal gradient ultracentrifugation, CsCl gradients, ultracentrifugation through sucrose cushion).

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Label

Label

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* * * Use Locations * * *

Use Locations

1. Will you be using or manipulating live animals on campus but outside of DCM Animal Facilities? If this is a Field Study, answer "No".* Y

Transportation of animals outside the central facility must follow guidelines set by IACUC.
Animal Transport Policy

Satellite Housing Location Detail (Housing outside of DCM Animal Facilities) is a Special Consideration.

Use Location Detail

Species	Building	Room	Activity
(b) (4)			
(b) (4)			
(b) (4)			
(b) (4)			

Use Location Detail

1. Species* Non-Human Primate (various)
2. Building* (b) (4)
3. Room* (b) (4)
4. Activity* Behavior Testing,Physcial Restraint,Physiological Testing
5. Why can't you do this in the DCM Animal Facility?

Requires specialized equipment.
We will also do these same activities also in East McDonnell Facility, room 00316A and East McDonnell Facility, room 00317A (eProtocol system does not allow me to add these locations separately).

Use Location Detail

1. Species* Non-Human Primate (various)
2. Building* (b) (4)
3. Room* (b) (4)
4. Activity* Imaging

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5. **Why can't you do this in the DCM Animal Facility?**
   Requires specialized equipment

Use Location Detail

1. **Species*** Non-Human Primate (various)
2. **Building*** [redacted]
3. **Room*** [redacted]
4. **Activity*** Imaging
5. **Why can't you do this in the DCM Animal Facility?**
   Requires specialized equipment

Use Location Detail

1. **Species*** Non-Human Primate (various)
2. **Building*** [redacted]
3. **Room*** [redacted]
4. **Activity*** Imaging
5. **Why can't you do this in the DCM Animal Facility?**
   Requires specialized equipment

*** Special Considerations ***

Special Considerations

Animal Re-Use: Procedures Performed on Animals Prior to Use on this Protocol

1. **Have any of the animals undergone procedures prior to being used on this protocol?*** Y

   Specify which animals underwent procedures, what procedures were performed, and where those procedures were performed.

[redacted] (rhesus macaque): had 6 procedures (head post implantation; recording chamber implantation; artificial dura implantation; viral injection; pial peel; chamber removal), and a minor external wound margin repair. Besides the head post implantation, these past procedures were all limited to one chamber location over the left primary visual cortex. The animal has been used for research after the head post was implanted in [redacted]. Invasive recordings stopped after the recording chamber over the left visual cortex was removed in [redacted]. These procedures were performed at the [redacted]. These procedures were done to develop an optogenetics approach with a transparent artificial dura to depolarize neurons in the visual cortex while the animal is doing a task. At WUSTL, this project will continue by using this optogenetic approach in a recording chamber positioned over the right extrastriate visual cortex, to understand the activity of cortical neurons in the segmentation of a visual scene into objects and background. The estimated number of surgeries that this animal will undergo at WUSTL is 3 (right hemisphere chamber implantation, artificial dura implantation, chamber removal). Viral injections will be done in a non-surgical procedure under anesthesia. Additional surgeries may be necessary to repair, modify or remove the implants.

[redacted] (rhesus macaque): had 1 survival surgery (headpost implantation) and 1 non-invasive head implant repair. All procedures were performed at the [redacted].

[redacted] (rhesus macaque): animal that will also be transferred from the [redacted]. This animal did not have any major survival surgeries.

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Two additional animals (rhesus macaque) are planned to be ordered from (b) (4) . These animals have had a fine needle aspiration/biopsy from their inguinal lymph nodes as part of an HIV vaccine study at (b) (4)), but they have not had other surgical procedures.

Food Restrictions or Regulations

2. Will you be restricting food or regulating food schedule?* N

Food Regulation/Restriction Policy, including Fasting for Procedures

Note:

This includes but is not limited to Nutritionally Incomplete Diets.

This does not include pre-surgical fasting.

Water Restrictions

3. Will you be restricting Water?* Y

Fluid Regulation/Restriction for Rodents or Fluid Regulation/Restriction for Large Animals

Water Restriction

Species	Duration	Frequency
Non-Human Primate (various)	20 ml/kg per day for animals below 5 kg and 15 ml/kg/day for animals over 5 kg	daily up to 6 days per week

Water Restriction

1. Species* Non-Human Primate (various)
2. Describe the water restriction duration 20 ml/kg per day for animals below 5 kg and 15 ml/kg/day for animals over 5 kg
3. Frequency of Restriction daily up to 6 days per week
4. Justification for water restrictions*

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Behavioral training is accomplished using positive operant conditioning techniques involving fluid rewards (water or juice). Thus, restricted fluid access is essential in order to motivate animals to perform the specific behavioral tasks required in our experiments. Each animal is maintained on a controlled water intake schedule that is individually tailored to the specific animal. Water control is usually accompanied by some weight loss, which is carefully monitored by measuring the animals' weights at least weekly. Some animals may be sufficiently motivated by mild fluid deprivation, whereas others require more stringent control (up to 15% weight loss), particularly in the early stages of training. Several variables interact to determine the necessary level of control. First, difficult behavioral tasks require more stringent control than easy tasks. Many of our experiments require the animal to perform near psychophysical threshold, which requires more stringent control. Second, large animals can tolerate a higher percentage of weight loss than smaller animals. Third, it is often possible to relax the fluid deprivation somewhat once an animal becomes proficient at the required task. Thus, the proper level of water control is a multi–factored judgment and cannot be precisely quantified. However, we attempt to use the minimum level of fluid deprivation necessary to train animals and maintain a reasonable rate of data acquisition.

We take great care to administer the water control regimen as humanely as possible. Four aspects of our procedures help to achieve this goal. First, each animal is routinely given the opportunity to work for as much water as it desires during the course of an experimental session. Most experiments are terminated because the monkey is no longer thirsty and ceases to work. Although this can be frustrating to the experimenter, we quit when the monkey quits. Second, if the experiment has to be terminated before the animal stops working, the monkey receives supplementary water as needed to bring his total intake up to the average level at which he is usually sated. This level varies somewhat from animal to animal, but is usually in the range from 15–25 ml/kg. Third, averaged over the course of one week, the minimum amount is set at 20ml/kg/day for animals below 5kg and at 15ml/kg/day for animals above 5kg. The different criteria account for the fact that smaller animals generally require a larger quantity of fluid per unit weight compared to larger animals. Fourth, on every individual day, animals will always receive at least 5ml/kg, and on no more than 3 consecutive days the intake will be below 10 ml/kg. In general, animals are rarely restricted to the minimum intake level described above, and receive much more water on weekends and on days when no experiments are scheduled.

5. **Describe the health monitoring procedures (e.g., body weight, blood urea nitrogen, urine/fecal output, Fluid/fluid consumed), frequency of checks, and the method of ensuring adequate nutrition and hydration during the regulated period.**

   Animals will be weighed at least weekly and evaluated for signs of dehydration.

6. **Describe the criteria for removing the restriction**

   Animals with a weight loss of greater than 15% will be removed from the study and provided access to water. If signs of dehydration or excessive weight loss are observed, urine specific gravity is measured. The veterinary staff will be consulted if there is any concern regarding an animal's health. We put a great deal of effort into training animals, and we are highly motivated to keep them healthy and happy. Laboratory personnel will monitor and record the body weight and fluid intake volumes for animals on water restriction. Such records will be kept in a laboratory record for each animal and will always be available to the DCM veterinary staff.

Exception from Environmental Enrichment Policy

4. **Are you requesting an exception from the Environmental Enrichment policies? A description of standard enrichment is available in the Help text*** N

   "Environmental Enrichment" is the process of providing stimulating environments for animals in order for them to demonstrate their species-typical behavior, to allow them exercise control or choice over their environment, and to enhance their well-being.

   IACUC policies (see policy links below) require provision of Environmental Enrichment for all species. If your animal cannot have the enrichment described, click Add to provide details and justification.

   Mouse and Rat Environmental Enrichment Policy

   Exercise and Environmental Enrichment for Dogs Policy

   NHP: Nonhuman Primate Socialization and Environmental Enrichment Policy

Exception from Social Housing Policy

5. **Will you need Single Housing for experimental reasons other than those single housing situations that are already pre-approved in the Single Housing policy?*** N

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"Social Housing" is defined as housing social species in compatible pairs or groups with additional visual, auditory, olfactory, and/or tactile contact with conspecifics housed in the same room.

IACUC Social Housing policy, Social Environment and Enrichment Policy

If your animals will not have the social environment described, click Add to provide details and justification.

Restraint of Conscious Animals

6. Will you be physically restraining a conscious animal? If you are only using brief, hand-held restraint for less than 5 minutes, please answer "No".* Y

Since prolonged physical restraint may be stressful to animals, all physical restraint, other than routine manual restraint, must be described in the animal protocol. Moreover, animal restraint must be limited to the minimum time required to achieve the scientific objective(s). Physical restraint for longer than 4 hours must be justified for consideration by the IACUC. Convenience alone is not adequate justification to use prolonged restraint. When restraint is required for more than 24 hours, consideration must be given to using the least restrictive method possible.

Restraint Policies:

Physical Restraint of Unanesthetized Animals

NHP: Acclimation of Nonhuman Primates to Experimental Restraint

Note: Include only prolonged restraint; brief restraint or restraint of anesthetized animals need not be described.

Restraint of Conscious Animals

Species	Restraint Device	Duration of Restraint	Frequency of Restraint
Non-Human Primate (various)	Primate Chair	Long-Term	Daily up to 6 days per week

Restraint of Conscious Animals

1. Species* — Non-Human Primate (various)
2. Restraint Device — Primate Chair
3. Restraint Type — Long-Term
   Short-Term = 4 hours or less
   Long-Term = Greater than 4 hours
4. Duration of Restraint — Up to 8 hours
5. Frequency of Restraint — Daily up to 6 days per week
6. Justification for using the restraint

   Data collection for this type of research involves recording eye movements in response to visual and other stimuli. Animals must remain stationary in order for data to be collected.
   Animals are trained to sit in primate chairs and execute visual guidance tasks (hand and/or eye movements) while viewing a computer-controlled stimulus monitor or projector screen placed in front of them. Each animal will be trained to move between its cage and the primate chair guided by a pole that attaches to a collar that it wears. In general, only a rigid pole will be used when personnel handle conscious macaques.
7. Describe the monitoring procedures and acclimation process

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Animals are trained with the pole/collar method by first getting them used to the pole. This is first done by placing the end of the pole inside the cage so they can touch and smell the pole. We place treats on the pole to encourage tactile exploration. This is usually done 1-3 times and they are holding onto the pole or perched on it. Next we hook the pole to the collar and let them get used to it while they are inside the cage. Treats are offered while the pole is on and they are inside the cage. Once they stand calmly with the pole on the collar inside the cage, we open the door and guide them to the floor and walk them in the room several times. We will drop treats on the floor while walking them to give them positive reinforcement. Once they are used to walking in the NHP room on the pole, we then guide them into their primate chair. Once in the chair we will offer them treats or juice by syringe as a reward for being inside the chair. They are acclimated to the chair by keeping them inside it longer each session until 45 min is reached. This entire process usually takes 1-4 weeks. Once the animal has a cephalic implant and has recovered sufficiently, they begin acclimation to head restraint. We begin with restraining and cleaning around the implant edges at first and giving treats or a juice reward. Once we begin training them to do our visual tasks, they are restrained for as long as they are willing to work which may initially be anywhere from 15 min to 1 hour. Then we increase the time as they become used to the task.

8. **Describe the criteria for removing the restriction, If restraint period is longer than 4 hours, describe the monitoring procedures and criteria for removing animals that do not adapt or acclimate to the restraint.**

The primate chairs, which leave arms, legs and trunk free, are adjustable along multiple dimensions to allow a normal and comfortable primate sitting posture. The animal's head is restrained using the head post during training and recording sessions to allow for eye-position monitoring and stable single-unit recordings. The head post does not put pressure on the skin and the head bar which holds the head post is adjustable to allow for a comfortable sitting position.
Animals are slowly conditioned to head holding. Initially, the head is restrained for brief periods of time. Once trained, animals willingly step into the primate chair and hold their head still while being fixed in place. This suggests that the animals tolerate the chair and head restraint well. We have never seen or heard from other investigators of lesions or illnesses. However, if an animal fails to adjust to restraint or shows signs of physical (lesions) or emotional distress, it will be removed from the procedure involving the restraint.

Satellite Animal Housing

7. Are you housing live animals outside of DCM Animal Facilities?* N

Proposed housing of animals outside DCM facilities for greater than 24 hours (aka, ÃÅa satellite facilityÃÅ) will be reviewed by the IACUC on a case-by-case basis and may be allowed only when adequately justified on a scientific basis. Approval is protocol specific and dependent on following the relevant policies below.

Standards for Housing Rats and Mice Greater than 24 Hours Outside DCM Facilities
Housing and Care of Zebrafish in Satellite Facilities
Prohibition for Bringing Non-IACUC Approved Animals into Surgical or Housing Facilities
Husbandry of Xenopus Laevis

* * * Non-Standard Husbandry * * *

Non-Standard Husbandry

Use the table below to describe any changes to standard care procedures including: bedding, cage change interval, light cycle, temperature, humidity, food type, water additives, caging, oxygen, hand sanitation, enrichment, food treats, etc]

Are you requesting any Non-standard husbandry or Care?* N

* * * Duplication & Alt. Search * * *

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Alternative Search

1. Duplication of Results

X I attest that the proposed animal activities do not unnecessarily duplicate previous experiments, whether my own or another investigator's experiments.*

Literature Search & Alternatives

2. Literature Search for Alternatives to Painful or Distressful Procedures.

If your protocol contains only Pain Class C procedures, check the box below. If you have Pain Class D or E procedures, enter your alternative search information in the table.

This protocol does not involve painful or distressful procedures (It has only Category C procedures).

Search Data

Search Date	Keywords	Databases Searched
06/06/2022	((fluid AND restriction) OR ("neuronal recording") OR ("animal welfare") OR ("animal research") OR (alternative) OR (multielectrode AND array) OR ("nitrous oxide" AND "general anesthesia") OR (radiographs) OR ("CAT scan") OR ("CT scan") OR (reduce AND number AND surgeries) OR ("artificial dura")) AND (monkey) AND ((neurophysiology AND perception) OR ("neurophysiological mechanisms" AND perception))	Pubmed

Search Data

1.	Search Range From*	1965	(YYYY)
2.	Search Range To*	2022	(YYYY)
3.	Search Date*	06/06/2022	(MM/DD/YYYY)

Note: Because this is a search for alternatives to painful or distressful procedures, you are advised to use the word "alternative" as a search term along with words that describe the painful procedures described in this protocol.

4. Keywords. Include painful procedures listed on the protocol*

((fluid AND restriction) OR ("neuronal recording") OR ("animal welfare") OR ("animal research") OR (alternative) OR (multielectrode AND array) OR ("nitrous oxide" AND "general anesthesia") OR (radiographs) OR ("CAT scan") OR ("CT scan") OR (reduce AND number AND surgeries) OR ("artificial dura")) AND (monkey) AND ((neurophysiology AND perception) OR ("neurophysiological mechanisms" AND perception))

5. Databases Searched*

Agricola Database	Alternatives to Animal Use in Research, Testing and Education
Animal Welfare Info Center	ATLA (Alternatives to Laboratory Animal Journal)
Benchmarks BioOne	BIOSIS

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CAB Abstracts		Current Contents	
CRISP		Google Scholar	
Lab Animal		Lab. Animals Journal	
Lab Animal Welfare Bibliography (QL55L27311988) MEDLINE	X	Pubmed	
PrimateLit		Public STINET	
Quick Biblio. Series		REE	
SCOPUS		TOXLINE	
TOXNET		Web of Science	
Other			

3. Based on your literature search, are there alternatives to the potentially painful or distressful procedures that would be compatible with your experimental design? N

   If "yes", please explain why you are not using the alternative(s).

4. Alternatives for Category E Procedures

   For Category E procedures, explain why pain relieving drugs or other treatments cannot be used to alleviate pain/distress.

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* * * Euthanasia * * *

Euthanasia *

Click Add to select the appropriate euthanasia method from the picklist. Any alternate methods need to be approved by the DCM Veterinarians.

Euthanasia

Species	Method of Euthanasia (primary)	Agent Name	Dose	Method of Euthanasia (secondary)
Non-Human Primate (various)	Barbiturate overdose	Pentobarbital Euthanasia Solution	more than 150 mg/kg	Exsanguination

Euthanasia

Species*	Non-Human Primate (various)
Method of Euthanasia* Primary	Barbiturate overdose
Describe Euthanasia Method	
Agent Name	Pentobarbital Euthanasia Solution
Route of Administration	Intravenous (IV)

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Justify the use of death as an endpoint rather than humane euthanasia based on criteria that indicate morbidity.	
Anesthesia Agent Name	
Route of Anesthesia Administration	
Dosage (in mg/kg if possible) or if inhalation or immersion agent, the concentration	more than 150 mg/kg
Method of Euthanasia (Secondary)	Exsanguination
Describe secondary method	Transcardial perfusion with saline and formalin
Explain why the animal(s) are not being euthanized.	

*** Funding ***

Funding

All funds supporting this protocol should be listed in this section if they will support animal activities performed under this protocol. If your funding agency or sponsor is not listed in the drop down menu, contact the IACUC office [314-362-3229 or iacuc@wustl.edu]

By adding NIH funding to this protocol, the PI confirms that all activities described in the grant application are approved on an IACUC protocol.

Funding - Grants/Contracts

Sponsor Name	Award Title	Status	Principal Investigator
National Eye Institute/NIH/DHHS/NEI	Border ownership and grouping in primate visual cortex	Pending	[redacted]

Funding - Grants/Contracts

1.	Sponsor Award Number/Grant #	R00EY031795
2.	Sponsor Name *	National Eye Institute/NIH/DHHS/NEI
3.	Award Title*	Border ownership and grouping in primate visual cortex
4.	Status	Pending
5.	Principal Investigator	[redacted]

Dept. Funding

Department Name	Award Title	Status
Neuroscience (003021)	Startup funds	Approved

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Dept. Funding

1.	Department Name*	Neuroscience (003021)
2.	Award Title*	Startup funds
3.	Status	Approved

*** Attachments ***

Please attach tables, diagrams, or other support documents that provide direct support for this protocol. Name all attachments and reference those names in the appropriate textboxes throughout the protocol.

Acceptable Attachment formats are: MS Word, MS Excel, MS PowerPoint, MS Visio, PDF, GIF, TIF, JPEG.

To update or revise any attachments, first delete the existing attachment and then add the revised document to replace it.

Document Name	Document Name	Attached Date
IBC Protocol (Biosafety and Chemical Safety)	[redacted] Approved IBC 220817	08/18/2022

*** Guidelines ***

Acclimatization and Quarantine	AGREE
Toe Clipping	AGREE
Aseptic Surgical Technique	AGREE
Genetically Modified Animals	AGREE
Rodent Cage Space and Weaning	AGREE
Use of CO2 Euthanasia in Rodents	AGREE
Testing of Cell Lines and Biological Materials	AGREE
Use of Pharmaeutical Grade Compounds	AGREE

*** Certifications ***

Statement Concerning the Care and Use of Laboratory Animals

- I will conduct the project in accordance with all applicable regulations including the PHS Policy on Humane Care and Use of Laboratory Animals, the Federal Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and published WashU IACUC Policy.
- I have determined that the research proposed is not unnecessarily duplicative.

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- Experimental procedures described in the grant application(s) associated with this protocol are congruent with procedures approved in this protocol and/or collaborating protocols.
- I accept responsibility for the conduct of all personnel on this protocol.
- All individuals working on this protocol have been assessed for health risks and have access to an Occupational Health and Safety Program (participation form is available here).
- All individuals working on this protocol will complete required institutional training courses before working with animals. Further, I certify that individuals assigned to perform specific procedures approved on the protocol are properly trained in those procedures, or will receive such training prior to working with animals.
- I will notify the IACUC regarding any unexpected events, complications, and unanticipated pain or distress that negatively affects animal welfare.
- I will consult with the veterinarian before providing any medical or emergency treatments not described in the approved IACUC protocol.
- I will obtain approval from the IACUC before initiating any changes to the approved animal protocol. Such changes include personnel, animal usage/housing locations, additional animals, modifications or additional procedures.
- I understand that work performed without IACUC approval must be reported to the IACUC and may be subject to federally mandated non-compliance reporting requirements. If a non-compliant activity is supported by the NIH, the expenditures or funds associated with the non-compliance must be returned to the sponsor.

X **I have read and certify that the above statements are truthful to the best of my knowledge.***

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* * * Event History * * *

Event History

Date	Status	View Attachments	Letters
06/14/2022	NEW FORM CREATED		
06/30/2022	NEW FORM SUBMITTED		
07/01/2022	NEW FORM REVIEWER(S) ASSIGNED		
07/05/2022	NEW FORM Comments Received (Cycle 1)		
07/05/2022	NEW FORM Comments Received (Cycle 1)		
07/07/2022	NEW FORM Comments Received (Cycle 1) - Completed		
07/07/2022	NEW FORM Comments Sent (Cycle 1)		
07/22/2022	NEW FORM Responses Received (Cycle 1)		
07/22/2022	NEW FORM PANEL REASSIGNED		
07/22/2022	NEW FORM Responses Sent (Cycle 1)		

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07/22/2022	NEW FORM REVIEWER(S) ASSIGNED
07/25/2022	NEW FORM REVIEWER(S) ASSIGNED
07/25/2022	NEW FORM Comments Received (Cycle 2)
07/25/2022	NEW FORM Comments Received (Cycle 2)
07/25/2022	NEW FORM Comments Received (Cycle 2)
07/25/2022	NEW FORM Comments Received (Cycle 2)
07/26/2022	NEW FORM Comments Received (Cycle 2)
07/26/2022	NEW FORM Comments Received (Cycle 2)
07/26/2022	NEW FORM Comments Received (Cycle 2)
07/28/2022	NEW FORM Comments Received (Cycle 2)
08/01/2022	NEW FORM Comments Received (Cycle 2) - Completed
08/01/2022	NEW FORM Comments Received (Cycle 2) - Completed
08/01/2022	NEW FORM Comments Received (Cycle 2) - Completed
08/01/2022	NEW FORM Comments Sent (Cycle 2)
08/03/2022	NEW FORM Responses Received (Cycle 2)
08/04/2022	NEW FORM PANEL REASSIGNED
08/04/2022	NEW FORM Responses Sent (Cycle 2)
08/04/2022	NEW FORM REVIEWER(S) ASSIGNED
08/04/2022	NEW FORM Comments Received (Cycle 3)
08/05/2022	NEW FORM Comments Received (Cycle 3)
08/20/2022	NEW FORM Comments Received (Cycle 3) - Completed

Protocol Title: Mechanisms of visual perception, attention and visually guided behavior in the primate brain

08/22/2022	NEW FORM Comments Sent (Cycle 3)		
08/22/2022	NEW FORM Responses Received (Cycle 3)		
08/22/2022	NEW FORM Responses Sent (Cycle 3)		
08/22/2022	NEW FORM REVIEWER(S) ASSIGNED		
08/22/2022	NEW FORM Comments Received (Cycle 4)		
08/22/2022	NEW FORM Comments Received (Cycle 4)		
08/22/2022	NEW FORM Recommended for Approval		
09/01/2022	NEW FORM APPROVED	Y	Y

Approved and Adopted: May 2015
Last Reviewed: June 2021

Washington University in St. Louis

Nonhuman Primate Socialization and Environmental Enrichment Policy

IACUC Policy:

This policy addresses nonhuman primate (NHP) social grouping, NHP environmental enrichment (EE), considerations of NHPs requiring special attention, the use of restraining devices for NHPs, and exemptions to the NHP EE plan. The policy has been developed with the involvement of the Attending Veterinarian (AV) and the IACUC to ensure environmental enhancement adequate to promote the psychological well-being of the NHPs used for research purposes at Washington University (WU). This policy will promote NHP well-being by working towards the goal of maintaining healthy animals free of physiological or psychological abnormalities, as well as acknowledging the responsibility of those concerned with animal welfare to provide animals with the most appropriate environment possible.

This policy is in accordance with currently accepted professional standards as cited in professional journals and reference guides, and as directed by the AV of WU.

Background:

Section 3.81 of the Animal Welfare Act (AWA) states that, *"research facilities must develop, document and follow a plan for environmental enrichment adequate to promote the psychological well-being of nonhuman primates (NHP)."*[1] At a minimum, the AWA mandates that the NHP EE plan address the following points: 1) NHP social grouping; 2) NHP EE; 3) NHPs with special considerations; 4) the use of restraint devices for NHPs; and 5) the exemption of certain NHPs from the EE plan. In addition, the *Guide for the Care and Use of Laboratory Animals* (*Guide*) (pp. 63-65) includes a Behavioral and Social Management section which describes potential enhancements to the cage environment, social environment, and activity level of animals, including NHPs, used in research.[2]

Non-captive NHP species have well developed social behaviors and hierarchies due to their natural lifestyles and habitats that include living in pairs or groups. Since captive NHPs are required to live in an artificial environment (i.e., primary caging), it is important to enrich the artificial environment to allow the animals to engage in species-typical behaviors that are believed to promote their psychological well-being.

Guidelines:

1. Behavioral Assessment:
   a) All macaques are assessed for behavioral abnormalities or maladaptive behaviors daily. Any physical or behavioral abnormality is immediately reported to a DCM veterinarian for appropriate action/treatment.

b) The DCM animal carestaff is also instructed to immediately contact the DCM veterinary staff if they have any concerns regarding the health and well-being of any macaque.

c) Documentation for the environmental enrichment of macaques is typically done by the DCM NHP veterinary technicians and includes:

i) **Primate Enrichment Log Sheets:** to periodically document the observations of the behavioral status of individual macaque.

ii) **Special Considerations Enrichment Log Sheets:** for individual macaques requiring extra enrichment for 'special considerations' (as described below)

iii) **Monthly NHP Enrichment Calendars:** documenting the types of enrichment given to the macaques in each of the animal housing room.

iv) **Monthly NHP Toy Calendars:** documenting the types of toys provided for each of the macaques in each of the animal housing rooms.

v) **Monthly Forage Board Calendars:** documenting the rotation of the forage board for the macaques in each of the animal housing rooms.

2. Social Interaction: The NHP species currently housed at the (b) (4) f (b) (4) facilities are rhesus macaques (*Macaca mulatta*) and cynomologus macaques (*Macaca fascicularis*). There is also a small marmoset (*Callithrix jacchus*) breeding colony at the (b) (4)

a) NHPs at the WUSM facilities are used primarily in research that involves conscious restraint of individual animals for behavioral, neurophysiological, and Parkinson's disease experimentation. Animals are also used for brain imaging and diabetes research.

i) PI and lab personnel will have access only to rooms where their animals are housed.

ii) Animal room assignment is based on proximity to the lab for the following reasons: 1) to ensure personnel safety; 2) to ensure animal safety and reduce animal stress; and 3) to limit personnel access for maintaining building security as well as animal biosecurity.

iii) EE and socialization procedures will be limited to the room where the animal is housed.

b) Marmosets at the (b) (4) are used primarily for auditory neurophysiologic research and are housed together in one room.

i) Auditory (vocalization and hearing), visual, and olfactory communication are important factors for the social interactions between marmosets. Marmosets are housed together in a single room on the (b) (4) where they can see, hear, smell, and vocalize with other marmosets.

ii) Marmosets are typically group-housed in families or pair-housed. Some marmosets may be singly-housed during the post-operative period or when a suitable partner cannot be identified; however, the goal is to find at least one compatible partner for each animal.

c) All NHPs at WU are socially housed with their own or compatible species with a minimum of visual, auditory, and olfactory communication. If possible, social housing will also include direct social contact for animals proven to be compatible in the same animal room.

i) Direct social contact allows species-typical behavior such as grooming. Pair housing animals in the same cage space is the highest form of social contact.

ii) Animals can be exempted from social contact on the basis of: 1) scientific justification by the PI that is approved in their IACUC protocol; 2) inability to find a compatible individual for social contact; and 3) the animal is exempted by the AV for medical reasons.

3. Environmental Enrichment: The AWA requires the enrichment of the physical environment by providing means of expressing non-injurious species-typical behavior and activities.[1] This can be accomplished by the use of cage complexities, inanimate objects to manipulate, varied food items, foraging or task-oriented feeding methods, and interactions with human personnel (i.e., DCM husbandry staff, DCM veterinary staff, laboratory staff). The AWA requirement for EE is satisfied in the following ways for the NHPs used at WU:

a) NHPs at the (b) (4) facilities:

i) Each cage will contain one permanently fixed perch situated in such a way as to not interfere with the cage squeeze mechanism and which will serve to increase the utilization of the cage space.

ii) Enrichment devices will be rotated at least every other week in all cages other than those housing animals in the "special consideration" category (see below) or those exempt from EE (see below). The use of these objects will be as documented in professional journals or resources, or as deemed appropriate by the AV.

iii) A variety of food items will be used during weekdays provided they are not limited by research protocol or veterinary advice. These will include a variety of fruits, vegetable, nuts, and commercially available treats.

iv) In addition to the regular diet, task-oriented feeding methods will be introduced regularly to prolong the useful enrichment time of particular food items and to help satisfy species-typical foraging behaviors.

v) Access to radio and television enrichment will be available to animals periodically during regular business hours on a rotational basis.

vi) Cage location within each animal room may be varied periodically to increase the diversity the environmental and communication between different animals within the room.

b) Marmosets at (b) (4):

i) Each marmoset cage will contain a nest box on a platform within the cage.

ii) Each marmoset cage will contain multiple branches to stimulate climbing and gnawing. In addition, the animals can climb and hang onto the plastic-coated mesh cage sides.

iii) A variety of food items will be regularly provided in simple devices or toys (e.g., PVC pipe pieces with holes or Kong toys) to mimic foraging behavior in tree limbs or placed directly in the gnaw grooves in the cage branches.

4. Special Considerations:

a) Section 3.81 of the AWA requires that, certain categories of NHP must receive special attention regarding enhancement of their environment.[1] These categories include:
   i) Infants and young juveniles;
   ii) NHPs that show signs of 'psychological distress' through behavior or appearance;
   iii) NHP used in research for which the IACUC approved protocol requires restricted activity (refer to IACUC Policy, "Acclimation of Non-Human Primates to Experimental Restraint");
   iv) Individually housed NHPs unable to see and hear other NHPs of their own or compatible species; and/or
   v) Great apes weighing over 50 kg.

b) All special considerations are documented in the Special Considerations Enrichment Log Sheets, as indicated previously.

5. Restraint Devices: Section 3.81 (d) of the AWA requires that, *"NHP must not be maintained in restraint devices unless required for health reasons as determined by the attending veterinarian or by a research proposal approved by the committee at research facilities. Maintenance under such restraint must be the shortest period possible. In instances where long-term (more than 12 hours) is required, the NHP must be provided the opportunity daily for unrestrained activity for at least one continuous hour during the period of restraint, unless continuous restraint is required by the research proposal approved by the committee at research facilities."* The IACUC acknowledges and adheres to the requirements concerning restraint devices as described above (refer to IACUC Policy, "Acclimation of Non-Human Primates to Experimental Restraint").

References:

1.) Animal Welfare Act (AWA). Public Law 890544. Title 7 of the U.S. Code (7 USC).

2.) The *Guide for the Care and Use of Laboratory Animals:* 8th Edition. *(Guide).* National Research Council. 2011.

3.) Lutz CK, Novak MA. 2005. Environmental Enrichment for Nonhuman Primates: Theory and Application. ILAR J 46:178-191.

4.) National Research Council (Institute for Laboratory Animal Research). 1998. The Psychological Well-Being of Nonhuman Primates. Washington (DC): National Academy Press.

5.) Nelson RJ, Mandrell TD. 2005. Enrichment and Nonhuman Primates: "First, do no harm". ILAR J 46:171-177.