

**PROTOCOL FOR ANIMAL USE AND CARE***Handwritten forms are not accepted***CRPRC**

EH&amp;S USE ONLY

**PROTOCOL # 10137****EXPIRES: 7/02/04**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone:		Phone:	
Fax:		Fax:	

Species (common names):	Number:	Source:
Rhesus macaque	6/year	CRPRC

Project Title: <u>Functional Properties of Neural Circuits for Vision</u>			
Overnight housing location::	Ctr for Neuroscience	Day use only:	Ctr for Neuroscience,
	Annex Bldg-Rm 124		Annex Bldg-Rms 119,124
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

**Procedures:** Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

We will record the electrophysiological activity of neurons in the visual thalamus and cortex of alert animals while they perform visual tasks. Individual animals will have a surgically implanted eye coil, head restraint post, and recording cylinder(s). For recording sessions, animals will be trained to sit in a primate chair and perform visually guided tasks for water or juice reward.

**Special Husbandry Requirements:** Describe any special requirements your animals have with respect to **food, water, temperature, humidity, light cycles, caging type, bedding**, or any other conditions of husbandry.

Water access is restricted. In practice, this means that the investigator will provide all the animals' water during the week, and the animal care staff will provide water on the weekends and holidays, as per investigator instruction.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input type="checkbox"/> Necropsy	

**Hazardous Materials** *(only if in the animal room):*

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	NIH/ McKnight Foundation	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	

**What Veterinarian or veterinary clinic will provide care for your animals? (check one)**

<input type="checkbox"/>	Lab Animal Health Clinic ( 2-0514 )	<input checked="" type="checkbox"/>	California Primate Research Center ( 2-0447 )
<input type="checkbox"/>	VMTH Large Animal Field Service ( 2-0292 )	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

*If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@ucdavis.edu) for current information about training and record keeping requirements.*

**Summary of Procedures:**

**a) Briefly describe the overall intent of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.**

Our goal is to determine how visual information is processed between the visual thalamus and cortex in alert animals and what effect visual attention plays in this processing. This basic research on information processing in the thalamus and cortex should further our knowledge of general mechanisms of cortical and thalamic function. The study of visual specializations unique to the primate will also add to our understanding of human vision.

**b) Procedures employed in this project:**

Please check the appropriate boxes if any of these procedures will be employed in your project:

<input type="checkbox"/> Monoclonal Antibody Production **	<input checked="" type="checkbox"/> Food or water restriction	<input type="checkbox"/> Special diets; food or water treatment.
<input type="checkbox"/> Polyclonal Antibody Production **	<input type="checkbox"/> Non-recovery surgical procedures	<input type="checkbox"/> Induced illness, intoxication, or disease
<input type="checkbox"/> LD 50 or ID50 studies.	<input type="checkbox"/> Survival surgical procedures	<input type="checkbox"/> Death as an endpoint (see i below)
<input type="checkbox"/> catheters, blood collection, intubation	<input checked="" type="checkbox"/> Multiple survival surgery	<input type="checkbox"/> Trapping, banding or marking wild animals
<input type="checkbox"/> Prolonged restraint. (8 hrs+)	<input checked="" type="checkbox"/> Behavioral modification.	<input type="checkbox"/>
<input type="checkbox"/> Fasting prior to a procedure.	<input type="checkbox"/> Aversive conditioning.	<input type="checkbox"/>

**\*\* If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.**

**c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)**

Because individual projects differ from each other only in terms of the types of visual stimuli shown to animals and/or the sites of neuronal recordings (thalamus or cortex), all animals can be considered to be in one study group. These animals are maintained for long periods of time, trained extensively on visual discrimination tasks, and often used in more than one related experiment. The procedures will be broken down into each constituent procedure in the following discussion.

**Surgical procedures.** All experiments require head restraint and the measurement of eye position, and these are performed according to well-tested methods originally developed by David A. Robinson. These methods are currently in use at a large number of laboratories within The University of California, across the United States, and abroad.

Under full surgical anesthesia (CRPRC surgical suite and support staff), we implant in one eye a 3-turn scleral search coil of fine, multistrand, Teflon-insulated stainless wire (method of Judge, 1980, with minor modifications). The leads for this coil exit the orbit laterally and run subcutaneously up to a dental acrylic head implant, which surrounds the eye coil connector (small plastic electronic plug), the head restraint post, and the recording cylinder. The entire acrylic implant is secured to the skull using a combination of transcranial, slotted orthopedic bolts and self-tapping orthopedic screws. The recording cylinder typically is located over occipital cortex, and covers a 2-cm craniotomy, formed using a hand trephine. Dura is left intact under the craniotomy, protecting the brain between recording sessions. Therefore, these procedures invade no body cavities. The Principal Investigator has extensive experience with all of these procedures, having performed them numerous times at Harvard Medical School before joining the faculty at UC Davis. He will be present during all surgeries.

*Multiple survival surgeries.* These are employed for three distinct reasons. First, animals often need extensive behavioral training prior to recording (see below). For these animals, the eye coil and head restraint post are initially implanted (these are necessary for training), and the recording cylinder or cylinders are implanted in a separate surgery after the animal is trained. This reduces any risks associated with the presence of the cylinder and reduces the daily maintenance of the animal during this period. Also, if a recording cylinder is left in place, the bone will typically regrow from the edges of the craniotomy. Thus, if the cylinder were initially implanted, a separate procedure would probably be required in any case, to reopen the craniotomy for recording. For similar reasons, a second cylinder is sometimes implanted in a second surgery, when recording will commence on the second hemisphere of each monkey. For some experiments it is necessary to simultaneously have access to both hemispheres, and for these animals, cylinders will be simultaneously present on both hemispheres. The second reason for a repeat survival surgery is the failure of an eye coil. These are designed to be flexible, and to move with the numerous, high-velocity eye movements that monkeys make. However, the wire of which the coil is made will eventually fatigue and electrical continuity will be lost, causing coil failure. For the continuation of the experiments, it is necessary to replace the coil. In practice, a coil will typically last about a year, but there is considerable variability (range about 6 months to 3 years). When a coil fails, there is usually no discomfort to the animal, so the coil is left in place, and a new one is implanted in the other eye. The last reason for a surgery is mentioned above: removal of bone regrowth from the edges of the craniotomy. Lastly, surgeries are sometimes necessary to either repair a damaged implant or to remove part or all of an implant if chronic infection develops underneath the implant.

The overall life history of an animal in the laboratory is controlled by several interacting factors: the condition of the cortex, the scientific necessity of recovering histological verification of recording sites, the continued need for behavioral data from the task for which the animal has been trained, etc. So, it is not practical to define in advance the total number of surgeries that each animal will receive, but it might be as large as six.

**Animal training.** Physiological measurements are made during two different behavioral tasks, different in difficulty and attentional demands. For both types of tasks, animals are trained to sit in a primate chair and watch a computer monitor. For some experiments, the animal need only be trained to maintain its gaze on a visual target presented on the screen immediately in front of the animal ("fixation"). This is natural behavior of a monkey, and is conditioned using standard operant methods and rewards of water or juice. In this process, desired behaviors (such as fixation) are encouraged by being paired with positive reinforcement. For this to work, the behavior being reinforced must occur spontaneously before training, and the reward offered must be desirable to the animal. The other behavioral task involves the animal making a sensory discrimination of stimuli controlled by the experimenter. Typically, these involve a categorical judgment of stimulus

type ("up" or "down"? ; "redder" or greener"?). In the course of training, each stimulus type is associated with a specific response alternative. In our lab, these responses are always eye movements to visual targets on the screen. Thus, in a typical experiment, the monkey might be presented with a single stimulus, moving up or down, and after the stimulus is presented, two targets appear, one above the stimulus location and one below. The monkey learns to make a rapid eye movement to the correct target to receive the reward. Incorrect judgments are followed by a brief time-out period, which approximately doubles the time interval until the next trial. In practice, the stimuli being discriminated are often very similar, to measure a threshold for sensory discrimination, and the animals typically work for approximately 75% correct performance, overall. To be useful for these experiments, the animals' performance must be crisp and reliable, and the measured thresholds must be repeatable across sessions. This requires extensive training on the specific task, prior to the commencement of physiological recording. A typical training sequence involves first fixation training (about 2-3 weeks), then the start of discrimination training. For most tasks, the animals learn the basics of the task in a few weeks, but getting asymptotically low and stable thresholds, generalized across different specific stimuli, requires several months of training. Training and recording sessions last 3-5 hours, 5 days a week (5 days/week is an upper limit). As an additional hour is necessary to transport animals from their cage to the laboratory, perform cleaning procedures on head wells, and configure equipment for physiological recordings, the maximum amount of time an animal will sit in a primate chair is 6 hours/day.

**Recording procedures.** All the experiments involve microelectrode recording from the visual thalamus and/or visual cortex, and these procedures employ "industry standard" methods widely used in the field. Specifically, we use the turret-and-grid system ( et al, 1988) to secure a removable nylon grid inside the recording cylinder, through which a 23 gauge stainless steel guide tube is inserted through the *dura mater*. On a daily basis, fine tungsten or platinum-iridium microelectrodes are inserted through the guide tube to record the activity of neurons in the target area. Between recording sessions, the guide tube is either removed or plugged with an antibiotic-coated wire. The guide tubes remain in a single location for up to a week at a time, and when they are moved, the grid and turret are removed for thorough cylinder cleaning. In addition, the cylinder is flushed every day through the holes in the nylon grid, using a dilute disinfectant consisting of Novalsan (1-2%) or Betadine (1%) in saline. Recording sessions last 3-5 hours (6 hour upper limit)/day, 5 days a week (5 days/week is an upper limit).

**Water restriction.** The scientific progress on these experiments is usually limited by the monkeys' work habits, since all data are collected from alert monkeys engaged in either simple (fixation) or complex (discrimination) visual tasks. Therefore, we are highly motivated to keep the animals in a maximally healthy and motivated state. Fluid reward is by far the most effective means of motivating the animals, and when monitored closely, provides no significant health risk to the animals. The exact level of restriction that is optimal is highly variable between animals, and also depends upon the difficulty of the task. Some monkeys will work for thousands of rewards per day with minimal restriction of intake if given desirable fluids; others will not work any better for fruit juice than for water. Therefore, the exact restriction employed for each animal is determined empirically in the first few weeks that an animal is in the lab on a daily basis. Much of that period is spent in adjusting the various parameters (type of reward, amount per reward, inter-trial interval, etc.) to jointly maximize the daily water intake and the daily work output from the animal.

We will follow the "Water Restriction Policy" (attached, and on the web at <http://ehs.ucdavis.edu/animal/waterrestriction3.html>) approved by the Animal Use and Care Administrative Advisory Committee (original statement approved 5/13/99, modifications approved 11/8/01). In general (full description attached), animals are divided into two groups: those under 8 kg, and those over 8 kg. Animals under 8 kg will be given a minimum of 150 ml/day, five days a week, and 225 ml/day, two days a week. Animals over 8 kg will be

given a minimum of 150 ml/day plus 10 ml/kg (for each kg over 8 kg) five days a week, and 225 ml/day plus 15 ml/kg (for each kg over 8 kg) two days per week. Optionally, animals may be given a constant daily water ration, with no added increment on the weekends, provided the total weekly amount remains the same as that given under the options above.

To verify the animals' physiology remains normal during water restriction, we monitor the following indicators of health and physiology, on a daily basis or as often as possible:

- 1) Body weight. The animals are weighed each day they are on study, on an accurate flatbed electronic scale. Modest weight losses (5-15%) are not unexpected; larger weight losses, especially if accompanied by any other signs, cause us to increase water dosage immediately. The baseline from which this weight loss is estimated is taken from the most recent weights shown by the animal when it was under *ad lib* water access.
- 2) Urine specific gravity. Whenever a fresh urine sample is available, we measure urine specific gravity.
- 3) Moistness of feces. This is one of the most useful and sensitive measures of hydration, in our experience, and fresh feces are always inspected.
- 4) Skin turgor. Folds of skin are inspected for resilience.

**Terminal procedures.** Animals will be euthanized at the end of the study, when we need for scientific reasons to verify our recording sites. To verify our recording locations, we make small "marking lesions". In addition, we will in some cases perform additional anatomical experiments tracing connections in thalamus and cortex with anatomical tracing compounds. Both of these anatomical procedures require good histology, which in turn requires perfusion of the brain at the time of euthanasia.

*Tracer injections:* Approximately two weeks before an animal is scheduled to be sacrificed, we will inject anterograde or retrograde tracers into physiologically identified thalamic and visual cortical areas. Injections will be made with a Hamilton microsyringe containing small amounts (0.3-0.5  $\mu$ l) of tracers, including fluorescent dextrans, lipophilic dyes, horseradish peroxidase, or biocytin. These tracers are picked up by neurons and are transported either to the terminals of the neurons (anterograde tracers) or back to the cell bodies of the neurons (retrograde tracers). We allow up to two weeks for tracer transport.

*Electrolytic marking lesions:* We may need to mark the boundaries of the region in which the physiological recordings have been made. For this purpose, we pass 10-20 microamps of DC current through the recording electrode in the region of interest. This causes thermal damage to a very small region of brain, approximately 100 microns in diameter, allowing us to recover the location of the electrode on histological sections. In each brain region from which we record, we will place between 3 and 10 of such marking lesions. These will be done in the last week of recording, after the tracer labels have been injected and immediately before the animal is sacrificed. The placement of seen such lesions in alert animals goes unnoticed by the animal, there is no pain involved.

*Euthanasia and perfusion:* Animals will first be tranquilized with Ketamine (10/mg/kg) and then euthanized with an overdose of sodium pentothal (100 mg/kg, IV) or sodium pentobarbital (80 mg/kg, IV). Once an animal has expired, it will be perfused in a fume hood (Annex room 124p) with a fixative (either paraformaldehyde and/or gluteraldehyde). The brain will be removed for anatomical studies.

**d) Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require**. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
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all	Surgery/training/restraint/water restriction	6	3
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### Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p><b>Examples:</b> domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.</p>
2	<p>Minor stress or pain of short duration</p> <p><b>Examples:</b> cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress</p>
3	<p>Moderate to severe distress</p> <p><b>Examples:</b> major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p><b>Examples:</b> exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.</p>

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

**Rhesus macaque (*Macaca mulatta*):** Visual processing in the rhesus macaque is thought to be remarkably similar to that in the human. For this reason, rhesus macaques are the most commonly studied species of macaque monkey and a vast amount of information exists for this species. Rhesus macaques are also extremely adaptable to restraint and easily adjust to conditions necessary for neuronal recordings.

Six animals will be required each year. This number is based on the number of animals required for a successful project. A successful project requires data from at least two separate animals. If a result appears variable across animals, then an additional animal is needed for the project. We plan to perform two projects a year, thus an upper limit estimate of the number of animals we will require is six (2 projects x 2(+1) animals).

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:  Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus	Ketamine	10	I.M.	Anesthesia induction
Rhesus	Atropine	0.05	S.C.	Anesthesia induction
Rhesus	Telazol	5-8	I.M.	Anesthesia induction
Rhesus	Isoflurane	0.8 - 2%	inhalation	To effect, 1-5 hr. (surgery)
Rhesus	Lidocaine	spray	topical	Once, to facilitate endotracheal tube placement

Rhesus	Lactated Ringers	5 ml/lb/hr	I.V.	continuous
Rhesus	Bupivacaine	0.5-2	IM	prior to all incisions and around wound margins
Rhesus	Keflin	40	I.V.	Once, per surgery
Rhesus	Cephazolin	20	I.M.	t.i.d. 5 days after surgery
Rhesus	buprenorphine	0.15	I.M.	t.i.d. 1-3 days @ vet's discretion
Rhesus	triple antibiotic ointment (ophthalmic)	~0.1 ml	topical	after eye surgery
Rhesus	Sodium Pentobarbital	80 mg/kg	IV	Euthanasia
Rhesus	Sodium pentothal	100 mg/kg	IV	Euthanasia

h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) **Adverse effects:**

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

1. Surgical recovery causes unavoidable distress.
2. Water deprivation. See attached "Water restriction policy" for details (also on the web, <http://ehs.ucdavis.edu/animal/waterrestriction3.html>).
3. Minor discomfort from guide tube insertion. The brain contains no nociceptors, and cannot feel the presence of the electrode, but the dura mater is richly innervated, and the guide tube passes through it.
4. Chronic implants. These are necessary for alert primate recording, and expose the animal to some risk of infection. Also, these implants occasionally suffer fractures, where either a recording cylinder or the head post breaks off from the dental acrylic.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

1. Post-surgical analgesics are given for 1-3 days.
2. Diet supplements, including Prima-treats, vitamins, and daily fruit are all given to ensure adequate nutrition. Periodic blood work-ups by the CRPRC also help to ensure the animals good overall health.
3. If the animals show any signs of distress, local anesthetics are topically applied prior to insertion of the guide tube.
4. The condition of the implants is monitored daily. The cylinders are flushed at least three times a week, and often daily, with saline and/or dilute disinfectants ("Novalsan", 1-2%; Betadine solution, 1%). All procedures comply with CRPRC S.O.P. II-33. If infection develops despite this prophylaxis, then veterinary assistance is sought. Typically, an X-

ray image is taken at the CRPRC (transport via CRPRC van) to evaluate whether the bone is infected, and cultures of any exudate are taken. Thereupon the best treatment is decided, and these can range from a course of systemic antibiotic to complete removal of the implant and prolonged recovery to allow for bone regrowth. In the event of a fracture in the implant, appropriate action is taken. If the fracture only involves acrylic, then repairs are made under ketamine anesthesia. If the fracture exposes covered tissue, then a temporary cap is fitted under ketamine anesthesia, and an emergency surgery is scheduled as soon as possible at the CRPRC to repair the implant.

*Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.*

Is death an endpoint in your experimental procedure?  Yes  No

*(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.*

**j) Literature search for alternatives and unnecessary duplication:**

*This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.*

What was the date on which you conducted this search?

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Medline (Medline is an all-inclusive search engine that searches <b>all</b> major databases)	1966-2002	visual & neurophysiology & method & alternative
Biosis	1993-2001	alternative & method & visual & behavior

What were your findings with respect to alternative methodologies?

The methodology outlined above is the industry standard that has used for the past 30 years. At present, there are no alternatives.

Has this study been previously conducted?  Yes  No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

**k) Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

1. When we need to recover the locations of recording sites for publication.
2. When the relevant areas of the brain have been sampled completely.

l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
<i>M. mulatta</i>	Overdose	sodium pentothal	100 mg/kg	i.v.
		-or-		
		sodium pentobarbital	60 mg/kg	i.v. or i.p.

m) **Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

No surplus animals are anticipated.



