

PROTOCOL FOR ANIMAL USE AND CARE
Handwritten forms are not accepted
CNPRC

EH&S USE ONLY
PROTOCOL # 10439
EXPIRES: 1/16/04

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

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

Species (common names):	Number:	Source:
macaque monkey	15/year	Primate Center
cat	20/year	ARS approved vendors
ferret	20/year	ARS approved vendors

Project Title Functional Organization of the Visual System

Overnight housing location::	Ctr for Neuroscience	Day use only :	Ctr for Neuroscience,
	Annex Bldg-Rm 124		Annex Bldg-Rms 121,124

Animals will be maintained by: Vivarium Investigator *(If investigator maintained, attach husbandry SOP's.)*

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.

Acute experiments will be performed on anesthetized animals in which 1) physiological recordings are made from the retina, thalamus, or neocortex, and/or 2) anatomical tracers are injected into the thalamus or neocortex. Animals will be euthanized and their brains will be processed for histology.

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.

Animals will be fasted (food withheld) on the day prior to surgery.

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

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

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

Infectious Agents?	[] Yes [x] No	Agent(s):	
Radioisotopes?	[] Yes [x] No	Agent(s):	
Chemical Carcinogens?	[] Yes [x] No	Agent(s):	
Toxic Chemicals?	[] Yes [x] No	Agent(s):	

Funding source:	NIH R01 EY13588	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8932

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

<input checked="" 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 the mechanisms by which visual information is transformed between the retina, the lateral geniculate nucleus (LGN) of the thalamus, and layer 4 of the primary visual cortex. This basic research on information processing in the cortex and thalamus 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 type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input checked="" 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 checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" 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.)

There is only one study group.

Acute physiological experiments (macaque monkeys, cats, and ferrets):

The goal of these experiments is to record the visual responses of neurons at different locations along the visual pathway (retina, thalamus, and cortex) of anesthetized animals. Doses given are for macaque monkeys and cats. Dosages for ferrets are the same, except as noted at the end of this section.

- The animal will be fasted (food withheld) the night before the experiment.
- Pre-medication. The animal will be given penicillin (300,000 units, IM) and dexamethasone (4 mg, IM) the day before the experiment. These doses will be repeated each day of the experiment.
- Initial anesthesia. Induction with ketamine (10 mg/kg, IM). Leg will be shaved and prepped and a catheter will be inserted into a leg vein. Sodium pentothal will be given IV (10 mg/kg) and supplemented as needed.
- The skin overlying the trachea will be shaved and prepped. A tracheotomy will be performed and a tracheal cannula will be inserted so that the animal can be ventilated during paralysis. All wound margins will be injected with lidocaine or bupivacaine.
- The animal will be placed in a stereotaxic apparatus. A heating pad will be placed around the animal and a rectal probe will be inserted. Temperature will be monitored throughout the experiment and adjusted accordingly. EKG leads will be placed on the animal and the heart rate will be monitored throughout the experiment.
- Maintenance anesthesia. Maintenance anesthesia will be either sodium pentothal (2 mg/kg/hr, IV), isoflurane (1-2%, inhalation) or a combination of sufentanil citrate (3-6 microgram/kg/hr) with isoflurane (0.4% inhalation). The sufentanil/isoflurane combination will only be an option for monkeys and will not be used for cats or ferrets. It should be noted that all 3 of these methods of anesthesia are currently approved for use in the laboratory. If physiological monitoring indicates a low level of anesthesia (see below), the maintenance rate will be increased. The animal will be monitored continuously throughout the experiment (this is possible since 2-5 people are working on each experiment, so that individuals can take turns sleeping). At no point will the animal be left unattended.
 - Under paralysis, anesthesia is monitored by several means. An increase in heart rate and a decrease in CO₂ are important signs of a decreased level of anesthesia, but the most reliable indication comes from the EEG. Desynchronization of the EEG (a sign that brain activity is fast and random-appearing, rather than slow and more periodic) is a clear sign that the animal is becoming alert. Conversely, spindle waves, well demarcated periods of very highly synchronized activity, indicate that the animal is anesthetized. Continuous spindling indicates that the animal is over-anesthetized. If desynchronization is seen, then anesthesia is immediately supplemented and the maintenance rate increased. This is a rare occurrence once the experiment is underway; the animals are quite stable once a proper dosage rate is found. Temperature, heart rate, CO₂, and EEG will be monitored throughout the experiment and recorded every hour.
 - To ensure that the criteria are adequate, in some experiments we will periodically withdraw the paralytic agent. This allows us to test depth of anesthesia by more direct methods, such as a withdrawal reflex.
 - The short half-lives of pentothal and isoflurane are in fact useful features of these drugs. If the animal is either over-anesthetized (as determined from the EEG, see above) or, more importantly, under-anesthetized (see above), corrections can be made rapidly.
 - There are alarms on the physiological monitors that alert the experimenter when a value (such as CO₂ or heart rate) gets out of range.
- Adequate caloric needs for the long experiments are met by mixing the paralytic in a solution of 2.5% dextrose in saline, for a total of 4 cc/kg/hr. Although this would not be adequate to maintain an animal for long in an awake state, in our experience, the amount of dextrose administered during the experiment is in fact sufficient. Metabolic needs are quite low, primarily since the animals are paralyzed.

- The skin overlying the skull will be shaved and prepped thoroughly. All wound margins will be injected with lidocaine or bupivacaine. Two electrodes will be placed between dura and skull for EEG monitoring.
- If the stability of microelectrode recordings is poor, it may be necessary to partially support the weight of the animal (cats and monkeys, not ferrets; the animal does not break contact with the table) by a clamp attached to a lumbar vertebra. This is achieved by making a small, 1 cm incision over a spinous process of an upper lumbar vertebra. All wound margins will be injected with lidocaine or bupivacaine. The muscle around the spinous process will be dissected and the spinous process will be clamped with a stainless steel instrument. The wound margins around the instrument are then sutured closed. When the weight of the animal is partially supported by this instrument, the movements caused by artificial respiration are greatly reduced. This is a common measure used by a number of laboratories to achieve stable microelectrode recordings *in vivo*. It is particularly necessary for experiments involving intracellular recordings. The surgery involved is quite minimal and is only performed under general anesthesia during terminal experiments.
- A craniotomy will be made over the occipital cortex and/or over the lateral geniculate nucleus (LGN) for microelectrode recording.
- The eyes will be dilated with 1% atropine. Contact lenses will be placed to refract the animal properly and to protect the eyes from drying.
- To minimize eye movements so that small receptive fields may be studied, the eyes will be affixed to flat metal pads that will be attached via posts to the stereotaxic apparatus. The pads will be glued to the sclerae (white part of the eye) just beyond the limbus with cyanoacrylate glue. This is a relatively common measure used by visual physiologists to insure that there are absolutely no eye movements, since some residual movements are present even under paralysis. Again, the surgery is quite minimal and only performed under general anesthesia during terminal experiments. Our experiments absolutely require well-stabilized eyes in order to study small receptive fields over a long period of time.
- For recordings in the retina, a guide-tube will be introduced into the orbit, via a hole in the pad affixed to the eye, and electrodes will be advanced through it. This is a common procedure to record from retinal ganglion cells and is currently employed by several laboratories.
- Paralysis. Once all surgical procedures are complete, the animal will be paralyzed with Norcuron (0.2 mg/kg/hr, iv) or gallamine triethiodide (6-8 mg/kg/hr, iv). Paralytic agents will be used only in conjunction with anesthetics and physiological monitoring (see above).
- After paralysis, the animal will be mechanically ventilated so that its expired CO₂ is maintained near 3.5%.
- If stability of microelectrode recordings is poor, a bilateral pneumothorax may be performed to minimize movements caused by artificial respiration. Again, it is a common procedure used by many laboratories to allow stable microelectrode recordings *in vivo*.
- Experiments will last for up to 72 hours for monkeys (up to 36 hours for cats and ferrets). During this time animals will not be left unattended. As described above, animals will be continuously monitored (EEG, CO₂, heart rate) for adequate anesthesia. The long duration of experiments is not uncommon in visual physiology; many laboratories (e.g., at Harvard Medical School, Rockefeller University, New York University and Washington University) routinely perform such experiments. Two factors influence the long duration of experiments: 1) several hours are necessary to correctly position electrodes prior to data collection, and 2) fewer animals are needed to collect sufficient data for a study.
- Euthanasia and perfusion. At the end of the experiment, the animal will be euthanized with an overdose of sodium pentothal (100 mg/kg, IV) or sodium pentobarbital (80 mg/kg, IV). Brains will either be removed for *in vitro* electrophysiology recordings or will be perfused with a fixative (formaldehyde, paraformaldehyde, and/or glutaraldehyde) and removed for anatomical studies.

Ferrets: Because of their size, ferrets are dealt with differently. Young ferrets can be quite small (120-400 grams for animals between 4 and 12 weeks). As a result, some dosages are significantly higher (per weight) than for cat or macaque. Also, experiments rarely last longer than one day. The induction dosage with ketamine is 100 mg/kg. Maintenance anesthesia is 10 mg/kg/hr sodium pentothal (IV). Paralysis is with 1 mg/kg/hr norcuron (IV). The dexamethasone and antibiotic doses are 1/4 those given to cats and macaques. Animals will be euthanized with an overdose of sodium pentothal (250 mg/kg) or sodium pentobarbital (200 mg/kg).

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
I	Acute Physiological Experiments (Ketamine, sodium pentothal, isoflurane, sufentanil citrate).	15 macaques 20 cats 20 ferrets	2

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: 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.
2	Minor stress or pain of short duration Examples: 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
3	Moderate to severe distress Examples: 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
4	Severe pain near, at or above the pain tolerance threshold Examples: 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.

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?

Macaque monkeys: Visual processing in the macaque monkey is thought to be remarkably similar to that in the human. In addition, color vision is best studied in the macaque monkey. Two types of experiments (*in vivo* and *in vitro* physiology) will be performed in the monkey. 15 animals will be required for these experiments. Past experience indicates that data from approximately 300 cells is necessary for reporting *in vivo* results in a peer-reviewed journal. Since data can be collected from approximately 20 cells/animal, 15 animals is a good estimate of the number of animals needed to collect the required data.

Cats: There is a large literature of cat visual physiology and the cat has become a model system for studying vision. Compared to the monkey, certain features of the cat visual pathway are more suitable for collecting certain types of data. In particular, the visual thalamus of the cat is 1) close to the surface of the brain, 2) consistently at the same stereotaxic location in the brain, and 3) flat as opposed to curved within the brain. All of these features make experiments that depend on locating a particular site in the visual thalamus easier in the cat, compared to the monkey. Similar to the monkey, approximately 300 cells are required to publish these types of results in a peer-reviewed journal. 20 cats @15 cells/cat should therefore yield the necessary 300 cells.

Ferrets: Compared to cats and monkeys, the visual system of ferrets is very immature at birth. The ferret is therefore an ideal choice for addressing questions about the development of the visual system. 20 animals will be required for these experiments. Past experience indicates that data can be collected from approximately 15 cells/animal. Since

publication of the results of this type of study generally requires data from approximately 300 cells, 20 animals is a good estimate of the number of animals that will be needed.

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?
Note: Surgeries are non-survival, therefore postop. procedures are not applicable				
Macaque monkey	Ketamine	10 mg/kg	IM	Initial induction
Macaque monkey	Sodium pentothal	2 mg/kg/hr	IV	Continuous (for maintenance anesthesia)
Macaque monkey	Isoflurane	1-2% (inhaled)	←	Continuous (for maintenance anesthesia)
Macaque monkey	Sufentanil citrate	3-6 µg/kg/hr	←	Continuous (for maintenance anesthesia); in conjunction with 0.4% isoflurane
Macaque Monkey	Norcuron	0.2 mg/kg/hr	IV	Continuous
Macaque Monkey	Sodium Pentobarbital	80 mg/kg	IV	Euthanasia
Macaque monkey	Sodium pentothal	100 mg/kg	IV	Euthanasia
Cat	Ketamine	10 mg/kg	IM	Initial induction
Cat	Sodium pentothal	2 mg/kg/hr	IV	Continuous (for maintenance anesthesia)
Cat	Isoflurane	1-2% (inhaled)	←	Continuous (for maintenance anesthesia)
Cat	Lidocaine or Bupivacaine	0.5-2 mg/kg	IM	prior to all incisions and around wound margins
Cat	Norcuron	0.2 mg/kg/hr	IV	Continuous
Cat	Sodium Pentobarbital	80 mg/kg	IV	Euthanasia
Cat	Sodium pentothal	100 mg/kg	IV	Euthanasia
Ferret	Ketamine	100 mg/kg	IM	Initial induction
Ferret	Sodium pentothal	10 mg/kg/hr	IV	Continuous (for maintenance anesthesia)
Ferret	Isoflurane	1-2% (inhaled)	←	Continuous (for maintenance anesthesia)
Ferret	Norcuron	1 mg/kg/hr	IV	Continuous
Ferret	Lidocaine or Bupivacaine	0.5-2 mg/kg	IM	prior to all incisions and around wound margins
Ferret	Sodium Pentobarbital	200 mg/kg	IV	Euthanasia

Ferret	Sodium pentothal	250 mg/kg	IV	Euthanasia
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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?

We will be making high-resolution maps of visual receptive fields. Any movement of the eyes will compromise these maps.

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

ECG, EEG, CO₂. In addition, the paralytic agent will be removed in some animals and withdrawal reflexes assessed to ensure that our criteria for determining proper depth of anesthesia are adequate.

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

Increases in the dosage of anesthetic agents will be given whenever the parameters listed above indicate that the animal is under-anesthetized.

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)

None. Prior to surgery, animals are food deprived for 1 day. Animals are anesthetized during surgery and will not recover from surgery. Although the experiments require the animal to be paralyzed, all surgical procedures are performed before paralysis is induced. To ensure animals are adequately anesthetized throughout the experiment, a number of parameters (see below) are measured. If these parameters indicate a low level of anesthesia, the delivery of the anesthetic agent is increased.

- Under paralysis, anesthesia is monitored by several means. An increase in heart rate and a decrease in CO₂ are important signs of a decreased level of anesthesia, but the most reliable indication comes from the EEG. Desynchronization of the EEG (a sign that brain activity is fast and random-appearing, rather than slow and more periodic) is a clear sign that the animal is becoming alert. Conversely, spindle waves, well demarcated periods of very highly synchronized activity, indicate that the animal is anesthetized. Continuous spindling indicates that the animal is over-anesthetized. If desynchronization is seen, then anesthesia is immediately supplemented and the maintenance rate increased. This is a rare occurrence once the experiment is underway; the animals are quite stable once a proper dosage rate is found. Temperature, heart rate, CO₂ and EEG will be monitored throughout the experiment and recorded every hour.

- To ensure that the criteria are adequate, in some experiments we will periodically withdraw the paralytic agent. This allows us to test depth of anesthesia by more direct methods, such as a withdrawal reflex.

- The short half-lives of the anesthetic agents—pentothal and isoflurane—are in fact useful features of these drugs. If the animal is either over-anesthetized (as determined from the EEG, see above) or, more importantly, under-anesthetized (see above), corrections can be made rapidly.

- There are alarms on the physiological monitors that alert the experimenter when a value (such as CO₂ or heart rate) gets out of range.

At the end of each experiment, animals (while anesthetized) will be euthanized with an overdose of sodium pentothal (100 mg/kg), IV.

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.

If the signs listed above indicate a low level of anesthesia, then the anesthesia is immediately supplemented and the maintenance rate is increased.

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?

12/11/02;
 1/14-03

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	1966-2003	Monkey, cat, ferret, vision, physiology, anatomy, cortex, LGN, retina
Personal collection of over 10,000 research articles	1900-2003	Monkey, cat, ferret, vision, physiology, anatomy, cortex, LGN, retina
Society for Neuroscience Annual Meetings	1992-2003	Monkey, cat, ferret, vision, physiology, anatomy, cortex, LGN, retina; attending paper presentations of latest research developments in the field
CRISP (NIH; Computer Retrieval of Information on Scientific Projects)	1972-2003	Monkey, cat, ferret, vision, physiology, anatomy, cortex, LGN, retina
Web of Science (Science Citation Index)	1975-2003	Monkey, cat, ferret, vision, physiology, anatomy, cortex, LGN, retina

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?

All animals are anesthetized during the duration of the study and euthanized at the end of the study.

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
Macaque monkey	Overdose	sodium pentothal	100 mg/kg	IV
		-or- sodium pentobarbital	80 mg/kg	IP
Cat	Overdose	sodium pentothal	100 mg/kg	IV
		-or- sodium pentobarbital	80 mg/kg	IP
Ferret	Overdose	sodium pentothal	250 mg/kg	IV
		-or- sodium pentobarbital	200 mg/kg	IP

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

All animals will be euthanized at the conclusion of each individual experiment.

