

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

EH&S USE ONLY

PROTOCOL # 9597**EXPIRES: _____****Investigator**

Last Name:		
First:		
Middle:		
email:		
Department:		
Phone / Fax:		
After hrs. #:		

Contact

Last Name:		
First:		
Middle:		
email:		
Department:		
Phone:		
After hrs. #:		

Species (common names):	Number:	Source:
Monkeys (<i>Macaca mulatta</i>)	60	UC Davis CA Regional Primate Research Center
Monkeys (<i>Callicebus moloch</i>)	10	UC Davis CA Regional Primate Research Center

Project Title	Effects of Sensory Deprivation on Adult Visual Cortex		
Overnight housing location::	Primate Center	Day use only :	Primate Center
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.

Under anesthesia, small amounts of a substance that blocks neural activity in the optic nerve is injected into one eye. After a survival time of a few days to a few weeks, the monkeys are euthanized and their brains used for neuronanatomical studies.
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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.

None

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 type="checkbox"/> Clinician to treat	<input 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 NS21377	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):	8131

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:	<input type="text"/>	Address:	<input type="text"/>
Day phone:	<input type="text"/>		<input type="text"/>
Emergency phone:	<input type="text"/>	Email:	<input type="text"/>

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.

The overall aims are:

To define how sensory experience affects gene expression in neurons that are concerned with the higher functions of the central visual system.

The Immediate questions and aims to be pursued have grown out of our original discovery that monocular visual deprivation in adult monkeys leads to alterations in gene expression for molecules associated with excitation and inhibition in the visual cortex.

The immediate aims are:

1. Examine the range of cortical neuronal molecules affected by monocular deprivation, using immunocytochemistry for selected transmitters, their receptors, transmitter-related enzymes, neuropeptides, and protein quinces, in situ hybridization for the related mRNAs, in monkeys in which retinal function is blocked unilaterally by tetrodotoxin (TTX).
2. Determine the magnitude of the effect by quantitative in situ hybridization histochemistry.

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 type="checkbox"/> | Non-recovery surgical procedures | <input type="checkbox"/> | Induced illness, intoxication, or disease |
| <input type="checkbox"/> | LD 50 or ID50 studies. | <input checked="" 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 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.)

This protocol will make use only of animals designated by the Primate Center for euthanasia for other reasons. (e.g. culling, old age, injury). There is a single experimental paradigm: in adult or juvenile monkeys under Ketamine anesthesia, the sodium channel blocker, Tetrodotoxin (15 micrograms in 10 microliters of saline), is injected into one eye. After a recovery period of predetermined length (1-84 days, but usually 1-4 days), the animals are euthanized under controlled conditions and various assays are made of the striate cortex on both sides. Normal undeprieved monkeys of the same body weight serve as controls. ("Controls" The controls have already been prepared by our supplies of normal brains from these two species, prepared in the same manner and stored in our freezers). All subsequent procedures are carried out on the dead brain.

Variations in the procedure are determined by the survival period required because a single Tetrodotoxin injection will only inactivate the optic nerve for 4 days. For survival periods of 1-4 days, a single injection will be given. For survival periods over 4 days, the animals will be reanesthetized at 4 day intervals and repeat injections given on each occasion. Because we will be using only animals designated by the Primate Center for euthanasia for other reasons, we anticipate that most animals will receive only a single injection but, where possible, we will use survivals of up to 12 days.

Because the administration of an anesthetic is always a potentially life-threatening procedure, the Tetrodotoxin injections must be regarded as surgery and when repeated must be regarded as "multiple survival surgery".

In each year, up to 12 monkeys will have TXX injections and one or two normal animals will serve as controls.

Virtually all assays to be performed can be carried out on the same animals, either on tissue from the visual cortex of the same side or by differentially utilizing the visual cortex of the two hemispheres.

Our principal objective is to freeze tissue at -70° for future assays of additional antigens, receptors or mRNAs, thus providing a brain bank for ourselves and other investigators to investigate the phenomenon of activity-dependent gene regulation. On average, approximately one animal per month will be processed. We cannot predict the exact ages of the animals to be used so the following table is a suggestion. That would provide a range of ages and survivals suitable for a statistically significant study of one major gene.

Detailed surgical protocol: Discomfort is obviated by ensuring that all surgery is carried out professionally, under anesthesia and under aseptic conditions. All surgical procedures are carried out by Dr. personally. His clinical training and his subsequent clinical practice involved training in general, neural and ophthalmic surgery, including blepharoplasty and corneal transplantation. Injections of Tetrodotoxin are made into the posterior chamber, through the conjunctiva and sclera, without any surgical incisions or suturing. The finest usable, beveled hypodermic needle (30 gauge) attached to a 10 microliter Hamilton syringe is used. Ten microliters of sterile normal saline containing 15 micrograms of Tetrodotoxin is injected over a period of approximately 3 minutes. Preoperative treatment involves routine health checks and overnight fasting.

Operations are always performed on one eye only, to avoid subsequent distress. For TXX injections, a surgical level of anesthesia is maintained with Ketamine since the procedure is very brief. If complications occur that necessitate more protracted surgical intervention (e.g. unusual bleeding), intravenous Nembutal will be added. We have never had any complications in these procedures.

Intubation equipment, respirator, suction and other resuscitative equipment is to be on hand at the table. A suite of rooms that meets the standards described in the NIH publication, "Guide for the care and use of laboratory animals" is used in the Primate Center. It is maintained in a state appropriate for aseptic surgery and animals are anesthetized and otherwise prepared in a separate anteroom.

Postoperatively, animals are given a prophylactic antibiotic and returned to their cages when fully alert. Further steps taken to prevent infection or secondary trauma include daily observation and cleaning, the avoidance of possible traumatizing objects in the cage and sacrifice at the first sign of any problems.

Bandaging or restraint is not used, because it causes the animals unnecessary distress. Induction of anesthesia usually takes 5-10 minutes, the surgery 3-5 minutes and recovery to consciousness ½ to 1 hour. Animals recover first in an incubator, maintained at 37°C and under constant supervision. When fully conscious, they are transported to their cages under continual supervision. The animals are visited at least once daily as routine. A log book is kept in the lab. After the survival period (two days to 12 days), the animals are anesthetized with IM Ketamine, given an overdose of Pentobarbital IV and perfused after the heart has stopped.

Euthanasia is determined by the fact that, as stated, the effect of a single TTX injection lasts 4 days. Hence the four day multiples. Within these, we will euthanize animals at the following time points to assess the effect of different periods of deprivation on gene expression. There are more animals in the longer surviving groups because effects are more overt and better quantifiable at periods greater than 4 days and because fewer genes show changes in expression at less than 4 days survival.

Note: Only brains from animals designated for euthanasia for other reasons are to be used. The numbers therefore are determined by availability. Also and very importantly this is not an investigation that stands alone but a continuation of brain collection that adds to material prepared over many years by this deprivation protocol and stored in our freezers. This material, we and others use to assess the effects of neural activity on gene expression, the genes to be investigated changing as new candidates are discovered. This is a unique tissue resource available to all investigators involved in studying activity-dependent phenomena in the nervous system. Because this is a tissue bank, the "group size" is not the group size of the animals designated here but the group size of all the brains collected over the years. In any investigation using this tissue the group size may vary depending on how many stored brains are used as the source of material.

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	Survival	Number of Animals	Category
(1) Macaca mulatta	Inject one eye	1 day	5	3
		2 days	5	3
		3 days	10	3
		4 days	10	3
(2) Macaca mulatta	Inject one eye at 4 day intervals for 8 days	8 days	10	3
		10 days	10	3
		12 days	10	3
(3) Callicebus moloch	Inject one eye	1 day	2	3
		2 days	2	3
		3 days	3	3
		4 days	3	3

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?

The use of 12-13 animals per year is the minimum sufficient to provide an adequate experimental series sufficient to gather enough data for statistical purposes. It is commensurate with our past experience of more than 30 years in determining what is sufficient for replicable results with this sort of material and for acceptance for publication by professional journals with high reviewing standards.

Since there are fewer *Callicebus* monkeys available, it is not possible to be assured of making repeat injections (necessitated as described by the 4 day duration of the TTX effect). Moreover because this effect has been studied to date in only a few *Callicebus* monkeys (by us) and only at the 4 time point, it is important to consolidate numbers at this time point into a statistically appropriate number (i.e as before numbers here plus numbers in our freezers).

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

Building:

Primate Center

Room:

Primate Center

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?
Monkeys	Ketamine	10 mg/kg	IM	Once – Induction of anesthesia
Monkeys	Pentobarbitol	60 mg/kg	IV	Once – Euthanasia
Monkeys	Oxymorphone	0-15 mgm/kg	IM	T.i.d. or p.r.m. – Analgesia

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?

Muscle tone, pupil size, heart rate, respiration, withdrawal from stimuli.

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

Any alterations of the above suggestive of lightening of anesthesia.

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)

Postoperatively, animals will not have a surgical wound. They will be blind in one eye with a dilated pupil on that side. The needle track could potentially become infected. This has never occurred in our past experience. If this occurs, the animal will be euthanized immediately. The visual loss may cause the animal to show some unwillingness to turn to one side but in our past experience has been insufficient to cause any distress. Repeated injections do not result in any added complications.

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.

Pain assessment, criteria and alleviation. A careful examination will be made daily for signs of impending infection, hemorrhage, or accidental or self-inflicted trauma. The first sign of incipient problems of this type will result in euthanasia of the animal. Signs of pain unassociated with infection or bleeding or self-inflicted trauma in the post-operative period include listlessness or agitation and withdrawal from stimuli, and if severe can be treated with analgesic. Because distress usually manifests itself by would manipulation, euthanasia is likely to be the treatment of choice.

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 [x] 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?

June 5, 2001

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
Personal (37,000 references)	1899-2001	cortex, visual, primates
Medline	1966-2001	cortex, visual, primates

What were your findings with respect to alternative methodologies?

I have considered possible alternatives to procedures that have the potential to cause pain and distress to the animals, and I have determined from my perusal of scientific journals and attendance at professional meetings over the last 35 years and from the Animal Welfare Information Center, that there are no less painful alternative procedures that would allow the same research goals to be achieved. Examination of activity-dependent phenomena that mimic those occurring in human disease or injury states requires the presence of an intact nervous system in which the brain is connected to the retina by the optic nerve and intermediate brain levels. In vitro preparations are therefore not feasible, and before data on the effects can be acquired, computational models are irrelevant.

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.

To provide a bank of brains in which the visual cortex has been monocularly deprived so that new activity dependent molecules, not examined in the original studies, can be investigated.

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

At conclusion of each experiment.

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
C. mulatta	Anesthetic overdose	Ketamine	10 mgm/kgm	Intramuscular
C. moloch		and Pentobarbital	60 mgm/kgm	Intravenous

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

There will be none.

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

Principal Investigator	Rank / Title	Date
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Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

Campus Veterinarian	Date
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