

PROTOCOL FOR ANIMAL USE AND CAREEmail to: campusvet@ucdavis.edu**CNPRC**

EH&S USE ONLY

**PROTOCOL: 10294
EXPIRES:**

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

Species (common names):	Number:	Source:
Rhesus monkey	10	CRPRC

Project Title	Developmental and Adult Auditory Plasticity		
Overnight housing location::	CRPRC / CFN	Day use:	CRPRC / CFN
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> 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.

Animals will be deaf in one or both ears. Brain activity will be recorded from a head implant on daily sessions.

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 restriction: some animals will be on the restricted water access protocol and should only be given posted amounts of water on weekends and holidays.

Food restriction: some animals will be on restricted food access and should only be given posted amounts of food on weekends and holidays

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 checked="" type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input 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:	N.I.H. (Pending)	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" 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 pcstillman@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.

Neuronal activity is well known to be critical in the development of the central nervous system. Two classic examples are the results of monocular deprivation on ocular dominance column formation in V1 and whisker ablation on the development of the barrel fields in S1 of rodents. These and subsequent studies have led to the general concept of 'critical' and 'sensitive' periods during development, in which afferent activity plays a major role in shaping the ultimate functional processing capabilities of the neocortex.

In contrast to the visual and somatosensory systems, very little is currently understood about auditory cortical development. Studies in non-primates have shown that there can be dramatic consequences of both unilateral and bilateral deafness in size, neuronal number, and physiological properties of both cortical and sub-cortical structures. Unilateral and bilateral deafness has been implicated in a number of auditory processing deficits in humans, such as sound localization ability and the usefulness of cochlear implants. However, we still do not know *which* changes in the auditory nervous system following neonatal deafness relate to perceptual and cognitive deficits. Further, there have been virtually no studies on auditory system development in non-human primates. The experiments of this protocol will extend to the macaque monkey the previous observations of changes resulting from neonatal unilateral and bilateral deafening made in rodents and carnivores. These studies will be further extended to assess the *functional* consequences of deafening assayed by sound localization performance, a fundamental auditory perception. We predict that unilateral deafness will produce sound localization deficits similar to those described in humans, and in contrast to other non-primate species such as the ferret. These results would provide further support for the usefulness of the macaque monkey as an animal model of human auditory perception. Further, we expect cross-modal plasticity to be observed in bilaterally deafened monkeys similar to those described in humans, where auditory cortical neurons come to respond to visual and/or somatosensory stimuli. The physiological and anatomical studies of this protocol will describe the underlying neurological substrates of this plasticity. It will also provide invaluable information on whether this plasticity is part of the functional recovery from neonatal injury, and should provide important insights into the general mechanisms and constraints of central nervous system development.

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 checked="" type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" 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.)

The objectives of this study are to define the neuronal activity at the single neuron level in auditory cortical areas in monkeys unilaterally deafened as infants and at 2 years of age and compare this activity to age-matched controls with normal hearing. Further, these properties will be related to the sound localization abilities of the animal. Finally, the neuroanatomical substrate of this plasticity will also be characterized. This entails deafening monkeys as infants or at 2 years of age. All monkeys will live at the CRPRC for two years after deafening, and then be transferred to the Center for Neuroscience and be trained to perform a sound localization task for food rewards. Monkeys will then be given MRI scans and single neuron physiological recordings will commence. Monkeys will be tested five days a week while single neuron activity is recorded. Following 9-12 months of recording monkeys will be euthanized and their brains prepared for histology.

Specific procedures:

MRI Imaging: In order to accurately place the recording cylinders, MRI images are taken of each monkey conducted by the CRPRC. These procedures entail transportation to the MRI facility, Metedomidine anesthesia (30-50 mcg/kg) under the supervision of CRPRC veterinarians and/or technicians. Monkeys are placed in a non-ferrous stereotaxic device, ophthalmic ointment is applied to each eye, and placed into the scanner. Each monkey will be scanned one time prior to the first surgery.

Recovery Surgeries: All surgeries will be conducted at the CRPRC surgical suite under the supervision of the attending veterinarian. I have been performing these and similar procedures for 7 years at the CRPRC without incident.

- 1) Animals are food and water deprived 12 hours before surgery
- 2) Atropine (0.5 mg/kg/s.c.) is administered 15 minutes prior to anesthesia
- 3) Anesthesia is induced with Ketamine (15 mg/kg).
- 4) An intravenous line is started, EKG leads are mounted, the animal is intubated, and the hair on the head and neck is removed. The animal is maintained on 0.8% - 1.5% Isoflurane and allowed to self-breathe. Lactated Ringer's solution is delivered i.v. throughout the surgery (5 ml/kg/hr).
- 5) A pre-op antibiotic is administered, as well as post-op antibiotics at the veterinarian's discretion.
- 6) The animal is sternally mounted in a stereotaxic frame, the surgical area is cleaned, prepped, and draped.

COCHLEOTOMY PROCEDURES:

An incision is made rostral to the ear canal and the skin and underlying fascia and muscle is retracted. The middle ear ossicles and cochlea are

exposed, in some cases requiring removal of the overlying bone. The ossicles are disarticulated by cutting the associated tendons and removed. The oval window is cut and the cochlear contents removed by aspiration. The muscle is sutured back into place. The skin is sutured and the wound closed.

NOTE: These procedures have not been performed by anyone in a macaque monkey to my knowledge. Prior to any experiments on live animals, cadaver animals will be dissected to develop the appropriate approach and acquire the necessary skill to perform the procedure adequately. In addition, I have developed a collaboration with Dr. and the MRC Hearing Research Institute in Nottingham (U.K.). Dr. has used this procedure on well over 100 animals to date (neonatal and adult guinea pigs and ferrets) and has agreed to assist me in developing these procedures.

HEAD IMPLANT AND RECORDING CYLINDER PLACEMENT:

An incision is made along the midline of the scalp and the skin, fascia, and muscle is retracted. Several titanium bone screws are implanted in the frontal aspect of the skull to support the head post. A craniotomy is performed over the temporal or occipital bone and several bone screws are implanted around the opening. The head post and recording cylinders are adhered to the skull using dental acrylic. Care is taken to ensure that the dental acrylic does not become too hot while it is curing by liberally applying sterile saline over the implant during curing. Depending on the results from the first experiments, it may be necessary to also investigate the cortical areas ipsilateral to the cochleotomy. If this is the case then two cylinders may be implanted in one procedure to avoid an additional surgery.

TRACER INJECTIONS INTO THE INFERIOR COLLICULUS:

An incision is made along the midline of the scalp and the skin, fascia and muscle is retracted. A craniotomy is performed over the occipital bone along the midline. The dura is cut and the cerebellum and cerebrum are gently retracted to expose the midbrain. A sterile sharpened Hamilton syringe mounted onto a micromanipulator is inserted into the inferior colliculus to a depth of approximately 3 mm. Wheat germ agglutinin conjugated to horseradish peroxidase is pressure injected into the inferior colliculus. The syringe is retracted. The brain is covered with a sterile contact lens. The craniotomy is closed with a pre-formed cap constructed of dental acrylic. The muscle and skin are sutured closed.

Multiple Surgeries: In some cases it may be necessary to repair an implant, either due to breakage, an infection, etc. In other cases it may be appropriate to implant only one recording cylinder and subsequently implant a second recording cylinder. Finally, it may be necessary to repeat the cochleotomy procedure if there is substantial recovery of hearing, or if it is deemed prudent to only lesion one cochlea in a given animal due to an adverse reaction to the anesthetic, etc. Each experimental animal will undergo at least one cochleotomy and one recording cylinder placement spaced approximately 2 years apart. In addition, animals that undergo unilateral cochleotomies will also undergo the injection of neuroanatomical tracer into the inferior colliculus. The rationale for these multiple surgeries is that there must be sufficient time for the developmental processes we are interested in studying to be completed after the cochleotomy (2 years). This way the animals are at no unnecessary risk of infection during this period. The recording cylinder must be implanted for the physiological studies, and will not be performed until after the behavioral studies are complete. The injections in the inferior colliculus must be made approximately 1 - 2 weeks before the animal is euthanized, or else the tracer will be absorbed and no longer visible. Under ideal conditions, an animal will be subjected to only two or three recovery surgeries during the course of these experiments. In the case of breakage, etc. this number may be increased, but these surgeries are not performed without extensive consultation with the CRPRC veterinarian staff.

Auditory Brainstem Response (ABR): These procedures are necessary to define the extent of the hearing loss after the cochleotomy. They will be performed before and after the cochleotomy procedure. In addition, ABRs will be measured at regular intervals to verify that no recovery is taking place (1 / 2 weeks to 1 / several months). Animals are anesthetized with

ketamine (15 mg / kg). Animals are laid onto one side and their head placed on towels or foam. A headphone speaker is placed over one ear. Thin stainless steel wires are placed through the skin by a 20 ga. needle behind the ear and at the vertex. Sounds of different frequencies (500 Hz - 20,000 Hz) are played at different intensities (10 - 90 db SPL). The recorded waveforms are averaged to determine the response of the auditory nerve and brainstem to these stimuli. If the animal begins to move or show other signs of being alert, anesthesia will be supplemented by 1/2 the induction dose. These procedures should be completed within 30 - 40 minutes.

Water restriction: Single neuron activity is recorded in monkeys while they are awake and with their heads restrained. These monkeys will not be performing an operant task at the time, but must be given some reinforcement to make the recording procedures a non-aversive environment and to maintain a steady state of alertness. Food rewards are inappropriate for these experiments as the chewing 1) produces sounds that will drive the auditory cortical neurons and 2) produces vibrations that will translate to the electrode causing injury to the brain. Procedures will adhere to the AUCAAC Policy Statement: Water Restriction in Rhesus Behavior Studies and all future amendments and revisions. Any deviation from these procedures will not occur until prior approval by the UCD AUCAAC.

Food restriction: The behavioral task (described below) will use preferred food rewards. Animals will be food deprived prior to the experimental testing (up to 22 hours). Small food rewards are given for correctly pressing the lever (see below).

Behavioral training/testing: The goal is to train the monkeys to stand on a platform in the center of a 16 speaker array. Once a characteristic orienting posture is acquired, an acoustic stimulus will be presented from one of the speakers. The monkey must walk to that speaker and press a lever located directly beneath it. If it is correct, a preferred food item (food pellet) will be dispensed and the animal is allowed to eat it. The monkey then returns to the platform to initiate another trial.

Initially, monkeys are acclimatized to the primate chair, laboratory, and laboratory personnel. Monkeys are then allowed to explore the apparatus and learn that depressing the levers dispenses the food pellets. Monkeys are then trained that food pellets are only available at locations that are emitting sounds. Finally, by the method of successive approximations, the monkey is trained to stand on the center platform to start a trial.

During routine testing, different acoustic stimuli (tones, broadband noise) will be tested at different stimulus intensities (25 - 80 dB SPL; barely audible to somewhat loud). Once the animal has ceased working and shows no inclination to approach the starting platform it will be removed from the apparatus. Supplemental food (fresh fruits and vegetables, nuts, raisins, etc.) will be provided, as well as monkey chow biscuits. These food items will be removed some time later. The goal of the food restriction is to maintain a motivated state for each animal, and will be adjusted to provide the maximum amount of calories for normal growth for each individual. Ideally, no food restriction will be necessary. While these procedures have not been attempted in monkeys, to my knowledge, colleagues have informed me that ferrets learn this protocol within 3-5 sessions (A.J. King, Oxford University) with 20 hours of food restriction.

MRI Imaging: In order to accurately place the recording cylinders, MRI images are taken of each monkey. These procedures entail Metomidine anesthesia (30-50 mcg/kg) under the supervision of CRPRC veterinarians and/or technicians. Monkeys are placed in a non-ferrous stereotaxic device, ophthalmic ointment is applied to each eye, and placed into the scanner. Each monkey will be scanned one time prior to the first surgery.

Recording Procedures: Electrophysiological recordings are made while the monkeys are passively listening to auditory stimuli. Each day the animal is transported to the laboratory in a primate chair and its head is fixed to the chair. The recording cylinder is cleaned as described in CRPRC SOP # 11-33: Maintenance of Chronic Cranial Implants. Briefly, the cap is removed and the cylinder flushed with dilute Nolvasan solution. A sterile cotton tipped applicator is used to scrub the inside of the cylinder. Lidocaine (20 mg/ml) and Nolvasan are placed in the cylinder (50:50) and left for at

least 10 minutes. The wound edge is treated with Nolvasan if necessary. The cylinder is flushed again with Nolvasan and the sterile grid insert is placed into the cylinder. A sterile guide tube is inserted through the grid, dura, and into the cerebral cortex. A sterile microelectrode is then inserted into the guide tube and the monkey is transported to the behavioral testing booth. The microelectrode is advanced remotely via a hydraulic microdrive until single neuron activity is encountered. The recordings then continue for several hours (generally 2-3 but up to as many as 7) until the monkey either becomes agitated, falls asleep, or stops drinking the water. In the event that the monkey does not receive its minimum daily requirement (for example, equipment breakage) the monkey is supplemented to its average working amount or the protocol minimum (20 ml/kg/day), whichever is greater.

At the end of the recording procedure the microelectrode is withdrawn from the brain, the guide tube and grid insert removed, and the cylinder flushed again with dilute Nolvasan. The monkey is given treats, the cap is rinsed with Nolvasan, and all fluid is aspirated from the cylinder. The cap is replaced, the monkey released from head restraint, and returned to its home cage for additional treats and enrichment.

Injection of neuroanatomical tracers: Different anatomical tracers will be injected into the cerebral cortex through the same guide tubes that are used for the electrophysiological recordings. Injections will be in the order of 0.1 - 0.5 microliters. The fluorescent tracers to be used are non-toxic at these concentrations (10% in sterile saline).

Euthanasia: At the end of all experiments, the monkeys are euthanized at the Necropsy suite at the CRPRC following the CRPRC SOP: Perfusing the Monkey. Briefly, the animal is deeply anesthetized until there is no longer a corneal reflex. An incision is made to expose the heart, the diaphragm is cut, the right atrium is cut and an infusion needle is inserted into the left ventricle. The monkey is perfused with normal saline followed by fixative (paraformaldehyde with or without 0.1% glutaraldehyde). These procedures are performed either by the Necropsy personnel at the CRPRC, myself, or a laboratory member trained by either myself or the CRPRC staff.

Numbers of animals: The scientific objectives of this study are dependent on recording from a large number of cortical neurons across a series of cortical areas. As these are also very valuable monkeys, most studies of this type use two animals in each experimental group, and two control animals, which are the numbers requested in this protocol. However, if there are considerable differences between the two animals in any given experimental or control group, a third animal may be required. In this case, an addendum to this protocol will be submitted. This study will entail four experimental groups (unilaterally deafened at birth; bilaterally deafened at birth; unilaterally deafened at 2 years of age; bilaterally deafened at 2 years of age) and one control group (normal 2 year old monkeys) making a total of 10 monkeys purchased over the first two years. Each experimental animal will be housed at the CRPRC for 2 years before behavioral testing (approximately 3 months) and physiological recording (approximately 9-12 months) procedures are performed. At the conclusion of these physiological studies, the animals are euthanized. A time line of when these animals will be used is appended to this protocol.

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
1	Unilateral deafening as infants	2	3
2	Bilateral deafening as infants	2	3
3	Unilateral deafening as 2 yr old	2	3
4	Bilateral deafening as 2 yr old	2	3
5	Normal 2-3 yr old animals	2	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?

(1) The macaque monkey is the ideal animal model for these experiments because (A) all psychophysical experiments performed on both humans and macaques germane to the experiments proposed have shown them to be comparable, therefore the macaque is a good animal model for human auditory perception, (B) these animals are very tractable and can be trained on the psychophysical tasks proposed and (C) the proposed experiments build on several previous studies conducted in my laboratory as well as others.

(2) Two monkeys per experimental group and 2 monkeys as controls are the very minimum necessary for statistical reliability.

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

Building:

CRPRC

Room:

Surgical suite

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 monkey	Ketamine	10 - 15	i.m.	Prior to surgery
Rhesus monkey	Atropine	0.05	s.c.	Prior to surgery
Rhesus monkey	Isoflurane	0.8-1.5%	Inhale	1-4 hrs / surgery
Rhesus monkey	Oxymorphone	10	i.m.	TID, 48hrs post surgery
Rhesus monkey	Keflin	40	i.v.	1 / surgery
Rhesus monkey	Buprenex	.005-.01	i.m.	TID
Rhesus monkey	Lidocaine	Spray	Topical	1 / surgery
Rhesus monkey	Metedomidine	30-50 mcg/kg	i.m.	Prior to MRI

Rhesus monkey	Lidocaine	1-3 ml of 2% solution	Topical in well	prior to electro-physiology recordings
Rhesus monkey	Antibiotic ophth. Ointment		Topical	1 / surgery
Rhesus monkey	Nembutal	40	i.v.	1: 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)

Animals will be deafened and housed with several peers in indoor/outdoor housing area. They may suffer injuries from other animals.

It is possible that the monkeys will develop an infection inside the recording cylinder or wound edge. This is monitored daily and the wound edge and cylinder is cleaned and inspected at least 3X week. Cylinders are cultured every 3 months. The animal may become dehydrated. Signs of dehydration include changes in affect, stool consistency, skin turgidity and urine color. Decreased growth rate is also a possibility. Adherence to the Restricted Water Access Policy Statement referenced above minimizes these possibilities. Animals may also show some weight loss during the restricted food access protocol. This will be detected as animals are weighed each experimental day. Weight loss of more than 5% over one week, or lack of weight gain over the course of 4 weeks will indicate inadequate growth.

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.

In cases of injury or infection the CRPRC vet staff is notified and the animal is treated at their discretion. For indications of dehydration the CRPRC vet staff is notified and the animal given supplemental fluids. For indications of weight loss or inadequate growth, animals are given supplemental high calorie foods.

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:

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

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit

http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu)

or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwood@ucdavis.edu)

What was the date on which you conducted this search?

8/01/02

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
Biosis	1993-present	Audit# Deaf# Anat# and Audit#, Deaf#, Physiol#
Bio85	1985-1993	Audit# Deaf# Anat# and Audit#, Deaf#, Physiol#
PsychInfo	1887-present	Audit# Deaf# Anat# and Audit#, Deaf#, Physiol#

What were your findings with respect to alternative methodologies?

These are state of the art methods and no alternatives exist.

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?

At the termination of the experiments, see summary of procedures (C) above.

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
Rhesus monkey	Cardiac perfusion	Pentobarbital	60	im or iv

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

All animals will be euthanized

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.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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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.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
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