

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

EH&S USE ONLY
PROTOCOL # <u>9488</u>
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:
Rhesus monkeys	12	CRPRC

Project Title	Scene Analysis in Auditory Cortex		
Overnight housing location::	CNS	Day use only :	ARS
Animals will be maintained by:	<input type="checkbox"/> Vivarium <input checked="" 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.

<p>Single neuron recording and reversible inactivation of auditory cortex are conducted on monkeys performing discrimination tasks. After 1-12 months of behavioral training, craniotomies are performed for implanting recording chambers and a post for fixation. During recording, the monkeys sit in a primate chair with their head restrained. The animals are placed on a restricted water protocol.</p>

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.

See attached instructions for animal care staff.
--

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 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:	NIH	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):	8117

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.

The objective is to determine cortical mechanisms underlying auditory perception. A more thorough understanding of these mechanisms will contribute to the treatment and control of many clinical disorders of the nervous system such as schizophrenia, stroke damage, dementia, aphasia, epilepsy, perceptual deficits, dyslexia, and language learning disorders. The results also have clinical significance for coding schemes for cochlear implants. The only presently available means to determine the brain mechanisms underlying perception is to investigate the brain directly. Electrophysiological and reversible lesion studies require the insertion of microelectrodes into the brains of animals and recording the neuronal activity that corresponds with behavioral performance. In order to correlate brain activity with the behavioral ability of the animal, it is necessary to perform these experiments while the animal is actively engaged in performing the behavioral task. Studies addressing this question cannot be performed in culture or in-vivo preparations because behavioral relationships cannot be established or the preparation is too reduced to permit a complete analysis of its function.

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 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 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.)

Overview:

The purpose of these experiments is to collect electrophysiological data from auditory cortex. Measurements from the brain are performed while the animal is performing a sound discrimination task, allowing for the establishment of a correlation between neuronal activity and perception. MRIs (see PG.4 'MRI Procedures:' for detailed methodology) are done on the monkeys to help identify the location of auditory cortex. We are still evaluating the usefulness of MRIs in identifying auditory cortex, because the anatomical location of the different cortical fields relative to sulcal pattern is highly variable. Then a series of three aseptic procedures follows. The first procedure is to implant a post for fixation of the head. (see pg.5 'Headpost Implantation:' for more details). The animal is given approximately two weeks to recover. After recovery, behavioral training (see pg. 5 'Behavioral Training:') is performed for 1-18 months. Once the animal reaches a criterion level of performance, the second procedure is performed. A craniotomy (see pg. 6 'Recording Chamber Implantation') is made in the left and/or right parietal bone and recording cylinders are implanted over each craniotomy. The dura is left intact and the chamber is sealed with a cap. A third procedure may be performed for the opposite hemisphere if this was not performed in the first procedure.

Determining if and when to expose the opposite hemisphere is dependent on several key variables which all relate to the scientific integrity of the experiments or the animal's well-being. The reason that this judgement call is necessary is because in every species studied the location of the auditory cortical fields cannot be solely determined on the basis of anatomy or cytoarchitecture. MRI provides slight improvement, but is still far from perfect. Rather, physiological mappings are required to identify the different cortical fields and their borders. For this reason we cannot unequivocally find the correct coordinates of the craniotomy and recording chamber. Because these animals will have up to a year and a half of training before any surgeries are performed they are very valuable to us, and missing the correct auditory cortical fields on both sides would be disastrous with regards to the experimental aims. On top of this, when both hemispheres are exposed, the risk of infection in the head increases because there are two openings. If we were only interested in recording single unit data, the ideal situation would be to record from one hemisphere and only after this was complete to expose and record from the other hemisphere. This would optimize the chance of getting large amounts of data from each hemisphere because the total time the cortex is exposed will be reduced. Also if the chamber were not in an ideal location in the first hemisphere we could slightly modify the placement of the chamber in the other hemisphere. However, a primary aim of these experiments is to determine the functional role of different cortical areas by reversibly inactivating different brain regions. If we were only collecting inactivation data and knew a priori the exact locations of our inactivation targets, it would make sense to expose both hemispheres at the same time. This is because there is a high probability that bilateral inactivation might have major effects on discrimination performance, whereas unilateral inactivation might show little or no effect. There are several critical parameters in determining how we proceed for each monkey.

- (1) How confident we are that we can place the chamber in the correct location. To a large degree, this will depend on the consistency of the MRI scans with other monkeys (if the MRI looks unusual we will have less confidence), and the level of success we have had with previous monkeys. We can never be completely confident because we know that variability of location of the auditory cortical fields exist between individual monkeys (e.g, see Merzenic and Brugge 1975)
- (2) How effective were unilateral inactivations in earlier monkeys. If unilateral inactivation prove highly effective, we will have an incentive to first collect some data on one hemisphere and then later open the other hemisphere to minimize the risk of infection and optimize the probability of getting complete data from both hemispheres..
- (3) How much data we have obtained from the other macaques in the study. As the project proceeds, we will need to fill in gaps where we don't have

enough data. If we need more single hemisphere inactivation data we will proceed with two step openings, whereas if we specifically need bilateral data we are more likely to try to open both sides in the same procedure.

(4) How well the initial procedure is progressing. We may enter planning a bilateral exposure. However, if the procedure does not go well (for example due to excessive bone bleeding) and progresses for a long time, we might opt to finish one craniotomy and do the other hemisphere at a different time. This judgement will be made depending on the risk to the monkey due to blood loss or extended anesthesia, and the higher risk of surgeon error over a longer, more trying procedure. In the past, these decisions have been made with the consulting vet at the time of procedure.

(5) How close we are to completely mapping out the other side of cortex. If we are performing two separate craniotomies, determination of when to perform the second craniotomy will depend on when we are convinced that we have mapped the other side completely. At that point we will know what, if any, minor adjustments we want to make on the placement of the chamber in the other hemisphere. This time scale is highly variable. If you look at combined data from our lab and the lab, this time can vary from several months to a year depending on the location of the auditory cortical fields relative to where we expect them to be, and how closely the organization of the cortical fields are to an "average monkey".

After recovery from a procedure (1-2 weeks) single unit recording and inactivation experiments are performed on a daily basis Monday through Friday for 1-5 hours each day. The animal is lightly restrained in a primate chair with head restraint via the head post. The animal is free to make postural changes and touch all parts of it's body (i.e. scratch) except it's head. Sterile stainless steel hypodermic needles (guide tubes) are inserted into the brain through the dura while the head is restrained and may remain in place for several days. During the period of restraint, recording electrodes are introduced into the brain through the guide tubes to measure the activity of single neurons. On some experimental days before single neuron recording, the pharmacological agent muscimol (or saline control) will be injected through the guide tube with a finer tube inserted through the guide tube, or a glass microelectrode, depending on the size of cortex to be inactivated. The muscimol will inactivate a small area of the cortex for 12-18 hours allowing us to assess the necessity of different cortical areas for auditory function and in generating clinically useful event-related potentials. The procedure involves no pain to the animal, no surgery or paralysis is performed and no anesthetics are used. Occasionally, the need arises for additional procedures to repair an existing implant. The experiments will last approximately 1 to 5 years.

MRI Procedure:

An MRI procedure will be used prior to implantation of the headpost and the recording cylinder. Our recording target region (auditory cortex) is located deep within cerebral sulci, and exhibits much individual variation with regard to depth, orientation and location, making precise positioning of our microelectrodes difficult. Obtaining a magnetic resonance image will permit us to target this region, with much greater accuracy than would otherwise be possible, by appropriately positioning the recording cylinder on the head.

In order to obtain an MRI which is aligned to the surgical stereotaxic plane, it is necessary to implant MRI-opaque beads onto the skull of the animal at a known stereotaxic coordinates. To do this, the monkey is first anesthetized with Ketamine HCl (8mg/kg), intubated with a tracheal cannula and an intravenous line inserted, and the head is shaved and cleaned. The monkey is then placed in a Kopf stereotaxic device and connected to a respirator permitting Isoflurane (1-2%) administration. A midline incision is made in the scalp and the fascia is reflected. Using predetermined stereotaxic coordinates, four glass beads (BE 3 mm diameter) filled with a 2% copper sulfate solution are attached to the skull using dental acrylic. The animal's vital signs are monitored throughout the procedure. Finally, the wound is closed in layers and the animal is recovered from anesthesia.

Following full recovery from the bead implantation, the monkey will undergo an MRI procedure. They will be anesthetized with Telazol (10 mg/kg) (or

appropriate alternative anesthetic on the advice of the attending veterinary staff), mouser in a Crist MRI compatible stereotaxic apparatus, and inserted into the bore of the magnet. T1 weighted, 3 mm thick coronal and/or saggital images will be acquired throughout the entire brain. The entire MRI procedure should take approximately 45 minutes. This procedure will not have any known adverse effects on the monkeys.

Headpost Implantation:

Atropine (0.05mg/kg s.c.) is administered 15 minutes prior to anesthesia induction.

Anesthesia is induced with Ketamine (15 mg/kg) and Acerpromazine (0.3 mg/kg).

Lubricating ophtalmic ointment is applied to both eyes.

An intravenous line is started, the ECG leads mounted, the animal intubated and a rectal thermometer inserted. The animal is maintained on 0.8 - 1.5% Halothane and allowed to self breathe. Lactated Ringers is delivered i.v. throughout the procedure (5 ml/lb/hr).

The top of the animal's head and neck is shaved and the animal's head is mounted in the stereotaxic frame and prepped for the procedure.

A pre-op and/or post-op antibiotic, such as Keflin, is administered. At the attending veterinarian's discretion.

The animal is then mounted sternally in the stereotaxic frame to allow implantation of the headpost. The area of interest is cleaned, prepped and draped.

An incision is made in the scalp and the periostium removed. Six to eight stainless steel screws are mounted in the anterior end of the skull and covered with acrylic. From one or two of the screws an electrical connection will be made and attached to a socket through the acrylic.

The wound is sutured closed using nylon suture.

A topical antibiotic, such as bacitracin, is applied to the wound margins and an ophtalmic ointment, such as BNP, is applied to the eyes.

A post-operative analgesic is given immediately following the procedure and every 12 hours for the following 48-72 hours (Buprenex, 0.01mg/kg) at the attending veterinarian's discretion.

Behavioral Training:

Prior to the first procedure the animal is brought to the laboratory and familiarized with the apparatus and primate chair. This is done by placing the animal in the primate chair and giving it ready access to the fluid reward and fruit while in the behavioral apparatus. This acclimatizes the monkey to the apparatus and investigator. The procedure lasts 30-60 minutes and is repeated 5 days/week until the animal is fully acclimatized (1-2 weeks).

Following recovery from the first procedure the animal is gradually familiarized with the head restraint. As before the animal is placed in the primate chair, given access to fluid and then mounted in the head frame. The head restraint is initially done for 3-5 minutes and increased in duration each day, 5 days/week, until the animal is comfortable with being restrained for at least 20-30 minutes (1-2 weeks).

Following the period of familiarization with the restraint the monkeys are trained on one of several tasks using standard operant conditioning techniques. In training they are rewarded with a small amount of water or juice delivered through a spout placed in from of the animal's mouth. Initially, the task is made very easy, and the difficulty is gradually increased to the shape the animal to the final behavior. The tasks animals will be trained on include several auditory sequence/pattern recognition tasks of varying difficulty. The total period trials/day and remain restrained for up to 5 hours without any evidence of discomfort or struggling. The end of a session is typically indicated by a drop in behavioral performance. If the animal struggles or vocalizes it is removed

from the restraint and the session is ended.

Attempts are made to train the animals on the behavioral task without resorting to water restriction. Those animals which perform poorly or not at all are placed on restricted access to water. Please see Supplemental Water Restriction Protocol Form (and CRPRC SOP# PP-1). Once the water restriction is initiated the animals' body weight is monitored daily. If the animal(s)' weight drops below normal or any signs of dehydration appear, the water deprivation is discontinued and the attending veterinarian will be contacted.

Each day the following will be checked to ensure the good health of the animals under restricted access to water: behavioral activity, general appearance, appetite, feces, urine and body weight. If the animals experience any illness or infection, their free access to water will be restored and the veterinarian will be consulted.

Recording Chamber Implantation:

All procedures are similar to those in the head post implantation.

Four additional skull screws are implanted around the margins of where the recording chambers will be placed.

A craniotomy is made in the parietal bone of the skull overlying the auditory cortex of either the right or left hemisphere, or both. A plastic or metal recording chamber(s) is(are) mounted over the opening(s) in the skull. The chamber(s) are fixed to the skull with dental acrylic. Once again, a sealed electrical connector may be connected to one screw via the cement from each hemisphere to allow ERP recording.

The chamber(s) are irrigated with sterile saline, a small amount of ophthalmic ointment, such as BNP, is applied, and sealed with a cap. The wound margins are sutured closed and the animal is recovered.

In case of structural failure of an implant or its attachment to the skull, we will need to immediately perform an emergency procedure to correct the problem because the animal will be more subject to health risks.

Electrophysiological recording:

Following recovery from the second procedure, electrophysiological recordings are made daily using the same behavioral procedures that were used in training. A plastic grid with 27 gauge hole placed at 1 mm intervals is attached to the interior of the recording chamber (Crist et al. 1988). An attachment to the recording chamber holds the grid in place and allows the entire assembly to be sealed with a cap. A sterile stainless steel 27 gauge hypodermic needle (guide tube) is inserted through this plastic grid into the cerebral cortex. This guide tube may remain in place for several days, and has a stopper to prevent fluid exchange between the cerebral cortex and the fluid within the chamber.

Immediately prior to each recording session, the monkey is placed in the primate chair, it's head is fixed, the cap is removed and the cylinder is flushed with sterile saline and hydrogen peroxide and/or a dilute betadine solution and/or Nolvasan solution. The wound edges are cleaned and disinfected with Betadine solution, hydrogen peroxide, or other disinfectants recommended by the veterinarians. The cylinder is then rinsed thoroughly with sterile saline and the cap scrubbed and cleaned. (Aggressive and regimented cleaning of the implant and surrounding regions will help prevent infections. See also CRPRC SOP# II-33.) The cap is replaced, the animal is released from the head restraint, and brought into the laboratory. The animal's head is fixed again, the cap and guide tube stopper are removed and placed in sterile saline with dilute antibiotic (Gentocin or other as recommended by the veterinarians). A sterile microelectrode is advanced into the brain through the guide tube with the use of one or more miniature mechanical micromanipulators attached to the recording cylinder. At the end of the session, the microelectrode is removed, the guide tube stopper replaced and the cylinder flushed with sterile saline. The cap is replaced and the animal is taken out of the restraint and returned to its home cage.

On days when muscimol is applied, the agent will be injected via a hamilton

microsyringe through one or more of the guide tubes or a pulled glass micropipette (et al. 1993). Muscimol (1 mg/ml) or saline will be injected in a volume of 1 ml at a rate of 0.1 ml/min. This concentration and volume of muscimol should evoke an effect lasting 12-24 hours (et al. 1993). The extent of inactivation will be measured with microelectrodes (1991; et al. 1993). Behavioral performance and neurophysiological recording will be performed after injection of muscimol.

Maintenance Procedure for Thinning the Dura Mater and Removing Excess Bone:

Due to bone growth, it is necessary approximately every 2-4 months to thin the dura mater and remove excess bone from the craniotomy. If this procedure is not routinely performed, the insertion of the guide tubes and electrodes becomes impossible. We anticipate most monkeys will be transported to the CRPRC for this procedure. However, animals requiring more frequent thinning (e.g. every other week) might have the procedure performed at the Center for Neuroscience Annex under the supervision of a veterinarian or an AHT (Animal Health Technician). If the monkey dura mater requires frequent thinning, the frequent transport of the monkey might interfere with its daily behavioral routine, and affect performance.

The animals are anesthetized with an i.m. injection of Ketamine (15 mg/kg) and Xylazine (1 mg/kg), and mounted in the stereotaxic frame using the existing headbolt assembly. The head area is cleaned, prepped and draped. The cap of the recording chamber is removed and the chamber is flushed with sterile saline and hydrogen peroxide. The layer of connective tissue overlying the dura is removed using forceps and a sharp periosteal elevator. The dura mater is thinned by removing the outer layers using forceps and a curved needle. Any excess bone that has grown back around the margins of the cranial opening is removed using a bone drill. At the end of the procedure, the chamber is rinsed with a disinfecting solution such as dilute betadine solution, and then with sterile saline. An antibiotic ointment such as BNP is put into the well, the cap is replaced and the animal is recovered.

Euthanasia:

All animals will be euthanized following completion of the electrophysiological studies. Anesthesia will be induced with Ketamine (15 mg/kg) and acepromazine (.3 mg/kg) i.m. Following anesthesia, a lethal dose of barbituate is administered (Nembutal 40 mg/kg) i.v. The chest cavity is opened and the pericardium is removed. Once the heart has stopped beating, the animal is perfused through the left ventricle with saline followed by fixative. This procedure is necessary to use the brain tissue post-mortem.

Monkey #26881

Monkey # 26881 ('Zeppo') presently is allowed to receive a minimum of 10 ml/kg, for a maximum of 1 day a week. This is only applied on a day in which he did not work for his minimum of 20 ml/kg. His weekly minimum remains at 260 ml/kg/week (average of 37 ml/kg/day) by making up the missed amount during the rest of the week (where the normal minima are applied). Without this, Zeppo routinely will not work for his minimum. We feel it is detrimental to reinforce this behavior by guaranteeing a supplement in his home cage, particularly when this protocol has worked on this monkey with no detrimental consequences. We have monitored this monkey carefully for any signs that this level of restriction is detrimental, including looking for excessive weight loss or hard stools, and have found no such evidence.

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	Behavioral performance with temporary cortical inactivation (using muscimol).	12	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?

Monkeys are the only lab animal species with the cognitive ability to perform the behavioral tasks necessary for this experiment. The number of animals is kept to the absolute minimum necessary to gain an appropriate amount of information. For these experiments it is necessary to run 6 different tasks of varying complexity for comparison purposes. About 100 penetrations (or fewer reversible lesions) can be performed in each monkey and at least 2 animals will be needed for each condition. We anticipate averaging two tasks per animal within this time frame, and therefore a need for 12 monkeys.

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

Building:

CRPRC

Room:

Surgical Suites.

Who will be the surgeon?

with a CRPRC Veterinarian

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?
Primate	Atropine	0.05	s.c.	Once, surgery induction
Primate	Ketamine	10-15	i.m.	Once, surgery induction
Primate	Acepromazine	0.3	i.m.	Once, surgery induction
Primate	Xylazine	1.0	i.m.	Once, surgery induction
Primate	Halothane	0.8-2.0%	inhalation	To effect, 1-4 hr. (surgery)
Primate	Lactated	5ml/1b/hr	i.v.	once, surgery

	Ringers			
Primate	Keflin	40	i.v.	Once, per surgery
Primate	Buprenex	0.005-0.01	i.m.	t.i.d. 1-3 hrs. @ vet's discretion
Primate	Lidocaine	spray	topical	Once, to facilitate endotracheal tube placement
Primate	Antibiotic Opth. Oint.	small amount	recording cylinders	when cleaned

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)

No undue signs of stress or discomfort should arise. The area around the headpost (craniotomy and skin) can get infected. Signs of infection are lethargy, leaky fluid, weakening of the headbolt, odor, and discoloration. Extra care is taken to observe possible dehydration or weight loss due to water restriction. Care is also taken to observe any potential infections in the head area.

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.

Post-operative analgesia will be administered and animals will be treated by CRPRC veterinary staff should signs of undue discomfort arise.

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?

4/22/01

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-present	monkey & vocalization & cortex
Medline	1966-present	monkey restriction alternatives
Medline	1966-present	monkey & awake & behaving
Psych	1940-present	Same keywords as above 3 on Medline

What were your findings with respect to alternative methodologies?

No other satisfactory methodologies were encountered.

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?

The animals will be euthanized when electrophysiology can no longer be performed or furthering the experiment would put the animal at health risk. Repeated penetrations into the brain and repeated use of muscimol at some point will compromise the cortical tissue to the point that recording is no longer feasible. We anticipate this to occur after about 100 penetrations.

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
Primate	Anesthetic overdose	Pentobarbital	88.0	i.v.

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

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>
--	------------------------------	----------------------

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>
-------------------------------------	----------------------