

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

EH&S USE ONLY

PROTOCOL # 9651**EXPIRES: 07/18/03****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 Monkey	8	CRPRC

Project Title Neural Correlates of Auditory and Visual Perception

Overnight housing location::		Day use only :	
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 are trained to perform sensory discrimination tasks for a fluid reward. After training, electrodes are introduced into the brain to record the activity of single neurons while they perform the task.

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

Animals are on the restricted water access protocol and should be given water by the animal husbandry staff as posted 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 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:	N.I.H.	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):	#8350

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 cerebral cortex is known to be involved in sensory perceptions of auditory, visual and combined auditory and visual stimuli. Several psychophysical studies in human subjects indicate that there are clear differences in the perception of these two stimulus modalities, with visual perception having much higher spatial acuity than auditory perception, whereas auditory perception has much greater temporal acuity than visual perception. For example, spatial acuity is normally dominated by visual input, such that if visual and auditory stimuli are presented simultaneously but from different (although nearby) locations, both stimuli are perceived to originate from the location of the visual stimulus (the 'ventriloquism effect'). Alternatively, when both visual and auditory stimuli are presented in different (but similar) rates at low frequencies (2 - 30 Hz), the rate at which visual stimuli are presented is perceived to be the same as that of the auditory stimuli (termed 'auditory driving').

While many studies have explored the neural correlates of visual perception, very little is understood about the neural correlates of either auditory perception or of the integrated perception of auditory and visual stimuli. One way to better understand how cortical neurons process auditory, visual, and combined auditory-visual stimuli is to measure the activity of single neurons in animals while they perform a behavioral task which allows a direct measurement of their percepts. Thus, the identical auditory stimulus can be perceived at a different location if a visual stimulus is simultaneously presented. Under these conditions, there should be single neurons that change their firing rates as a function of the percept, as opposed to as a function of the actual stimulus location. The same holds true for other neurons that are encoding the rate of presentation of visual stimuli, which will be perceived as either faster or slower if an auditory stimulus is presented at a faster or slower rate. These 'capture' effect may provide a valuable tool in determining how the responses of cortical neurons could give rise to both real stimuli and illusions.

This protocol describes experiments that will investigate the cortical areas and mechanisms of these fundamental perceptions and illusions. It is expected that at "early" levels of cortical processing, corresponding to primary and secondary cortical areas, cortical neurons will respond to the actual stimulus, and not be influenced by stimuli of the other modality. At "higher" levels of the cortical hierarchy, such as multi-modal areas in the temporal and parietal lobes, neurons are expected to have transformed their responses away from the physical stimulus

characteristics and toward the perception, including illusions.

The results of these studies should provide invaluable information on how cortical neurons integrate information across different sensory stimuli. A better understanding of these mechanisms should provide key insights into a variety of neurological maladies in which the inability to integrate information across sensory modalities has been implicated, such as learning disabilities, schizophrenia and autism.

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.)

The objectives of this study are to define the neuronal activity at the single neuron level in auditory and combined auditory-visual cortical areas and compare this activity to the animals perception. This entails training monkeys to perform a variety of different sensory discrimination tasks for fluid reward and then to implant chronic head restraint and recording devices. Monkeys are then behaviorally tested five days a week while single neuron activity is recorded. Monkeys are expected to participate in these experiments over the course of several years.

Specific procedures: All procedures outlined below have been routinely used by my laboratory over the past 5+ years and all have been previously approved by the UCD AUCAC.

Water restriction: Monkeys are trained by the method of approximation to discriminate different auditory and visual stimuli. 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.

Behavioral training/testing: The goal is to train the monkeys to make a behavioral response when they detect a change in a sensory stimulus for a fluid reward. Initially, monkeys are acclimatized to the primate chair, laboratory, and laboratory personnel. Monkeys are then trained to depress a lever, and then release the lever once an acoustic stimulus is presented (a broadband noise) for a fluid reward. Over the course of several weeks, the time from the lever being depressed until the acoustic stimulus is presented is extended. Other stimuli are then introduced from a different location, and the monkey learns to maintain the lever depressed during the stimuli presented from the first location, and to release the lever when the stimulus changes location. Over several weeks the distance between the speakers is reduced, and multiple "target" stimuli are introduced. At the end of training, the monkey will respond to ANY change in location of the noise stimulus. In this way it is possible to determine how far a stimulus location has to change for the monkey to detect the change. Monkeys are then trained to generalize across different stimulus types (noise, tones, vocalizations, etc.), stimulus intensities (20 - 75 dB SPL; very quite - loud conversational levels), and starting locations.

Most animals will also be fitted with a scleral search coil (see below) and trained on an eye movement version of the same task. Monkeys are initially rewarded for fixating a small LED illuminated in a dark room. The time that the monkey must fixate is gradually increased. Auditory stimuli are then presented either to the left or right of the midline, the fixation point is extinguished, and two target lights are presented. The monkey must then saccade to and fixate the target light that is closest to the acoustic stimulus location. In other experiments, the rate of flashing of an eccentric LED will change, and the monkey must then saccade to the target light that corresponds to "faster" or "slower", depending on the change in rate. Finally, future experiments may entail discriminating other features of auditory and/or visual stimuli, such as the direction of frequency modulated sweeps, stimulus intensity, the color or orientation of visual stimuli, etc. In our experience, monkeys can easily generalize to perform these sensory discriminations across different stimulus dimensions once fully trained on the task described in detail above.

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.

Recovery Surgeries: All surgeries will be conducted at the CRPRC surgical suite under the supervision of the attending veterinarian. I have previously performed all of the following procedures 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.
- 7) An incision is made along the midline of the scalp and the skin, fascia, and muscle 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.
- 8) Some animals will have a scleral search coil (eye coil) implanted. An incision is made in the conjunctiva at the intersection with the iris. The conjunctiva is bluntly dissected from the rest of the eyeball. The eye coil is implanted around the sclera and may be attached to the eye either by small sutures or cyano-acrylate. The leads are fed sub-cutaneously from the lateral aspect of the orbit up to the head implant, where the lead are soldered or crimped to an electrical connector. All exposed wires are covered with dental acrylic and become part of the head implant. Lubricating ophthalmic ointment is applied liberally to each eye.

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 the eye coil and head post for further training before implantation of the recording cylinder. Finally, it may be necessary to implant a second recording cylinder over the contralateral hemisphere in

an additional survival surgery. The rationale for these multiple surgeries is that the risk of infection increases with time once a recording cylinder is implanted. As there is a considerable time and energy investment in each animal during the behavioral training phase (which may take up to a year or more), it is best to limit the amount of time that a problem may arise. It is also best to implant a second recording cylinder in a well trained monkey as opposed to using a second animal. Under ideal conditions, an animal will be subjected to either 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.

Recording Procedures: Electrophysiological recordings are made while the monkeys are performing the behavioral tasks described above. 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 while the monkey performs a behavioral task outlined above. The recordings then continue for several hours (3 - 7) until the monkey stops performing the task, which usually occurs when it is no longer 'thirsty'. In the event that the monkey does not receive its minimum daily requirement (for example, equipment breakage) the monkey is supplemented to its minimum amount (20 ml/kg/day).

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.

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 the recording cylinders are relatively small compared to the area subtended by the regions of interest, it is not possible to record from all areas in a single cylinder. In addition, in spite of the MRI images, the functional boundaries of different cortical areas are not strictly defined by the pattern of sulci and gyri in the cerebral cortex, so individual variation may cause the cylinder to be placed differently in each animal. For statistical reliability it will be necessary to have at least two and preferably three animals in which any given cortical area is investigated, thus it will require six animals to completely explore all necessary cortical regions. Secondly, these experiments are critically dependent on animals that are able to sit quietly for several hours and perform the behavioral tasks reliably and with great vigilance. If animals do not perform well, for example by not continuing for the full daily ration of water, not attending to the stimuli, performing poorly or not at all on the "hard" trials, shaking the primate chair (which invariably kills the

neuron under study) or simply refusing to participate for unknown reasons, the animals is of no use for these experiments. While we have continued to work with other investigators and the veterinarians and staff at the CRPRC develop criteria to screen animals, it is not an exact science and it is never certain how a particular animal will react once it is restrained and psychophysical threshold data are collected. For this reason, it may well be that an animal is determined to be inappropriate for study after the head restraint and eye coil are implanted. Thus, I am requesting 8 animals (instead of 6) to compensate for this possibility.

Time Line: These experiments are run in parallel, and this protocol is a continuation of a previously approved protocol so there are monkeys currently under these procedures. I expect to have as many as 6 monkeys running at various stages of training / testing at any given time. With respect to an individual animal, the time line is as follows:

- 1) Obtain animal from the CRPRC, complete with current screening procedures and chair training.
- 2) Behavioral training (3 - 6 months)
- 3) Implantation of head restraint / eye coil
- 4) Continued behavioral training (3-6 months)
- 5) Implantation of recording cylinder
- 6) Behavioral testing / electrophysiological recordings (6 mo - several years).
- 7) Implantation of second recording cylinder and repeating step 6 above or euthanasia.

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	Adult Rhesus monkeys	8	3

Categories of invasiveness

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

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

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

(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) As stated above, it is necessary to collect data from at least two and preferably three animals for each cortical area. Since it is not possible to investigate all cortical areas of interest in each animal, at least six will be necessary. Two additional animals are requested in the event that some monkeys prove intractable for the behavioral tasks necessary or other unanticipated problems arise and are not able to complete the study. If this does not happen then these additional animals will not be used.

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)

It is possible that the monkeys will develop an infection. This is monitored behaviorally 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 behavioral affect, stool consistency, skin turgidity and urine color. Decreased growth rate is also a possibility. Adherence to the Policy Statement referenced above minimizes these possibilities.

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 case of infection or signs of dehydration the CRPRC vet staff is notified and the animal is treated at their discretion.

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?

6/30/01

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
PsychInfo	1887-present	Primate auditory physiology; visual
Medline	1966-present	Primate auditory physiology; visual

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 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	Nembutal	40	im or iv

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

All will be euthanized.

