

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

EH&S USE ONLY
PROTOCOL # 9734
EXPIRES: 09/12/03

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

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

Species (common names):	Number:	Source:
Rhesus	22 Aged	CRPRC
Rhesus	22 Young Controls	CRPRC

Project Title Estrogen and the Aging Brain, Animal Core

Overnight housing location:	CRPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

Procedures: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

This protocol is to maintain a colony of 22 aged animals and 22 young controls over three years for a program project studying estrogen, cognition, and the aging brain. This is a continuation of an ongoing NIA program project. Animals will be monitored daily for menses activity. Periodically animals will receive hormone replacement either by IM injection or subcutaneous silastic implants. A subset of animals will be euthanized for anatomical studies.

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.

Periodically animals will have urine collection from pans placed beneath their cages.

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 checked="" 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):	

Is the project already funded? Yes No
 Proposed Funding Source:

Previously approved? Yes 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:	<input type="text"/>	Address:	<input type="text"/>
Day phone:	<input type="text"/>		<input type="text"/>
Emergency phone:	<input type="text"/>	Email:	<input type="text"/>

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctlillman@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 goal of the program project is to determine what changes occur in neuroanatomy and cognitive behavior with the decrease in estrogen observed during menopause. The animal core will maintain 22 aged female macaques and 22 young controls and maintain records of the animals' menses cycles and hormonal profiles. Animals may also be anesthetized for ovariectomy to simulate post menopausal females. A subset of aged animals (N=16) and young animals (N=16) will be tested with a battery of behavioral tasks to assess attention and memory function. Animals will also be provided to other investigators in the program project at Mt. Sinai.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- | | | |
|---|--|--|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input checked="" type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see h below) |
| <input type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

Animals on this project will undergo the following procedures: Animals will have their daily menses activity monitored by direct observation. In addition, urine will be collected from pans placed beneath their home cages. Both of these procedures involve no animal manipulation. For specific program projects, subgroups of the aged animals will be chair trained in preparation for behavioral testing. All females will also be ovariectomized to simulate menopause. Animals will be anesthetized with ketamine 10mg/kg intubated and placed on isoflurane anesthesia. The animal will be given atropine .04mg/kg and receive a standard ovariectomy by CRPRC surgical staff. Following surgical recovery, animals will receive estrogen replacement therapy with either monthly 1.0ml intramuscular(IM) injections of a depo estrogen suspension used in veterinary medicine or a 3 cm silastic implant placed subcutaneously in a small skin incision between the dorsal scapulae. The total number of animals to be used over three years is 44; 22 aged females and 22 young controls. These animals will be used for four different research programs. A subset of aged females N=16 will continue behavioral testing. A second set of young control females N=16 will begin behavioral testing. One series of tests will use a Wisconsin General Testing Apparatus (WGTA). Animals are placed in a testing cage, 2½' X 4', and allowed access to a testing board through a barred window. The technician sits outside and tests memory function with food rewards.

Animals may be tested up to 3 hours but will have a technician present during that time. For testing of attention, animals will be placed in a standard CRPRC restraint chair and placed in front of an automated computer screen. By performing tasks on the touch screen, animals will receive food pellet rewards. Animals will perform the attention task for 2 hours or less. Animals will be tested on only one task at a time, five days a week. Total food provided to each monkey remains the same, although a smaller meal is offered in the morning, prior to testing. The remainder of the food is provided in the afternoon. Research technicians assigned to the project will feed the animals on days of testing to closely monitor overall appetite and behavioral performance. In year two, a short series of pharmacological challenges will also be conducted, aimed at testing the cognitive effects of cholinergic and N-methyl D-aspartate(NMDA) antagonist administration in relation to estrogen status. The behavioral assessment procedures will consist of a subset of the same learning and memory and attention tasks described above. On drug test days, monkeys will receive a single i.m. dose of the muscarinic receptor antagonist scopolamine (Sigma) or the NMDA receptor channel blocker MK-801 (Research Biochemicals), dissolved in sterile saline, 30 minutes prior to behavioral assessment. A short ascending dose series will be tested across days in each animal with scopolamine administered at doses of .32, 1.0, 3.2, 5.7 and 10 µg/kg⁻¹, and MK-801 doses of 3.2, 10.0, 17.8, and 32 µg/kg⁻¹. A minimum of two vehicle injection days will intervene between drug test sessions. By this design, less than 40 drug test days will be provided per subject over the two-year course of the test battery. A subset of 16 behaviorally tested animals (8 aged females and 8 young females) will be euthanized for anatomical studies upon completion of all behavioral tasks.

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	Urine Collection	44	1
2	Ovariectomy (16 aged females already ovx.,	25	3

	need to ovx additional 25 animals to complete the young behavior and histology groups)		
3	Chair trained (16 aged females are trained)	16	2
4	Hormone replacement/control	44	2
5	Behavioral testing	32	1
5	Scopolamine Dosage	32	1
5	MK-801 Dosage	32	1

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 the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

The number of animals in the animal core was determined by the experimental needs of the different projects in the overall grant. At any time point there will not be more than 22 aged and 22 young animals housed with this project. For the behavioral test (N=32) 16 animals will receive hormone replacement, 16 will receive placebo. Based on previous studies 8 is the minimum group size to detect difference in acquisition scores with unpaired two-sided student t tests and repeated measures ANOVA delay within subject.

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

Building: Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus	ketamine	10	IM	for immobilization

	isoflurane	inhaled	to eff	surgery
	oxymorphone	.75	IM	two days post-op.

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 may experience discomfort following ovariectomy. Animal may also develop a post-operative infection from ovariectomy or hormone implant.

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.

All animals will receive a minimum of two days post-op analgesia. Animals will be observed daily for post-op complication and treated by the CRPRC veterinary staff.

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?

9/01

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 Healthstar	1995-2001	Estrogen, Aging, Nonhuman Primate
Current Primate Reference	1970-1982	Review of printed bibliography

What were your findings with respect to alternative methodologies?

These types of studies have been conducted in rodent models but have not been performed in the nonhuman primate model. There is an extensive volume of literature on the role of estrogen in the hypothalamus and reproductive behavior, but very little in reference to cognitive performance.

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?

Animals will be euthanized for different program projects at time points following ovariectomy and hormone replacement.

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 Macques	Perfusion	Pentobarb	To effect for deep plane of anesthesia	IV

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

Return to aged colony.

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>
<i>CRPRC Director</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>
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Antibody Production Project Description

If your project involves only antibody production, either polyclonal or monoclonal, you may complete this page in lieu of section c), project description.

c) Will these animals be used for antibody production? Yes No

1. Polyclonal or Monoclonal antibodies?
 If Monoclonal, will you be producing ascites tumors in the animals? Yes No

2. What type(s) of antigen will be used?
 Will the antigens be sterile?

3. What adjuvant will be used for the initial injection?
 What adjuvant will be used for subsequent injections?

4. What route will be used for injections?
 What anatomical location will be injected?
 How many injections at one time?
 How frequently will injections be given?
 What volume will be injected at each site?

5. Polyclonal Blood collection Procedures:
 Who will collect the blood?
 From what anatomical location?
 How frequently will blood be collected? Volume?
 Will the animals be sedated? Yes No

6. Will monoclonal antibodies be produced in mice bearing ascites tumors? Yes No
 How often will the animals be assessed for abdominal distention?
 How often will they be tapped?
 How many times will they be tapped?
 Will the animals be sedated for tapping?

Note: If you are producing monoclonal antibodies using ascites tumors in mice, section i), alternatives, must explain why an *in-vivo* system is not suitable for your study.

7. Sedation / Anesthesia for blood or ascites collection: If the animals will be sedated for either injections or collections, please indicate the species, drug, dose and route:

Species	Drug	Route	Dose (mg / kg)
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

h) What criteria will be used to determine that the animals should be euthanized rather than continue to be used?

Categories of Invasiveness in Animal Experiments

Use these categories when completing item d), Study Groups and Numbers

Each year, the US federal government requires a report from the campus in which animal projects are categorized as to degree of invasiveness. Please assist the IACUC in this determination by assigning the animal procedures in your project to one of the categories below. The *US Government Principles Regarding the Care and Use of Animals* state, "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals."

1. Experiments which cause 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, but not intrathoracic or intracardiac (Category 2); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose or decapitation; short periods of food and/or water -deprivation equivalent to periods of abstinence in nature.

2. Experiments which cause 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; short periods of food and/or water deprivation which exceed periods of abstinence in nature; behavioral experiments on conscious animals that involve short-term, stressful restraint: short term exposure to noxious but non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters such as respiratory or cardiac rate, or fecal or urinary output, or in social responses.

3. Experiments which cause moderate to severe distress or discomfort

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, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of adjuvants which cause clinically evident swelling or abscesses.

Other examples include induction of anatomical and physiological abnormalities that will result in pain or distress: the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems.

Note: procedures used in Category 3 studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioral patterns or attitudes, the absence or grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.

4. Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals

Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress: completely new biomedical experiments which have a high degree of invasiveness; behavioral studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the American Veterinary Medical Association; any procedures (e.g. the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain

** The text of these categories has been freely adapted from a document originally published by the Canadian Council on Animal Care (CCAC).

tolerance threshold and cannot be relieved by analgesia (e.g. when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint).

