

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

EH&amp;S USE ONLY

**PROTOCOL # 9960**  
**EXPIRES: 6/19/04**

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

Species (common names):	Number:	Source:
Rhesus	24	CRPRC
Fascicularis	12	CRPRC

Project Title	Neuroanatomical studies of the Primate Limbic System		
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.

Rhesus and fascicularis macaques of various ages will be anesthetized and have one or more neural tracer substances stereotaxically injected into the brain. The macaques will recover, and, following a two-day to two-week survival period, they will be deeply anesthetized with pentobarbital and perfused. The brain will be processed to allow mapping of the injected tracer. Animals will receive injections of the non-toxic cell division marker BrdU several weeks prior to surgery in order to study the importance of newly generated neurons for brain circuitry.

**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.

no

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input 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 checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	Tritiated amino acids
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, NIMH, Macarthur Fndn.	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):	8550

**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 [ptillman@ucdavis.edu](mailto:ptillman@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.

A precise understanding of primate neuroanatomy is essential to the study of the neural basis of behavior. The goal of this project is to determine the connections of structures, specifically the hippocampal formation and amygdala, which are located in the temporal lobe. These data will provide information relevant to the understanding of memory mechanisms and the biological basis of primate social behavior. This information can be applied to studies of the mechanisms and potential therapies of various neurological disorders, including Alzheimer's Disease and Autism. These data are also the basis for behavioral and electrophysiological analyses that are conducted in Dr.            laboratory.

**b) Procedures employed in this project:**

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

- |   |  |  |
|---|--|--|
| <input type="checkbox"/> Monoclonal Antibody Production **        | <input type="checkbox"/> Food or water restriction               | <input type="checkbox"/> Special diets; food or water treatment.   |
| <input type="checkbox"/> Polyclonal Antibody Production **        | <input type="checkbox"/> Non-recovery surgical procedures        | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies.                   | <input checked="" type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below)        |
| <input type="checkbox"/> catheters, blood collection, intubation  | <input 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.)

Tracing of neural connections will be analyzed in mature and developing macaque monkeys. Animals will be sedated with ketamine (10-20 mg/kg) and receive one or two (24 hr interval) injections (i.v. or i.p.) of the non-toxic cell division marker BrdU (5-bromodeoxyuridine; 100 mg/kg) three to four weeks prior to tracer injection surgeries in order to evaluate the importance of newly generated neurons for the connectivity of the developing and mature animals. All animals will receive an MRI analysis of their brain prior to surgery. Neonatal animals will be transported, accompanied by a veterinarian to the UCDCM research MRI facility. There the animal will be anesthetized, placed in a stereotaxic apparatus, subjected to MRI, and transported without delay to the CRPRC and directly to the surgical suite. A scalp incision will be made followed by small burr holes in the skull with a dental drill. In addition to stereotaxic coordinates a microelectrode will be lowered into the brain to define the precise neuronal population for labeling. A micropipette will then be placed at the chosen coordinates to allow deposition of one of several tracers (PHA-L, BDA, Fluoro-Ruby, Fluoro-Emerald, Fast Blue, Diamidino Yellow, 3H-amino acids, CTB, WGA-HRP or similar neuronal tracers); following injection, the micropipette will be removed. Each monkey will receive up to five different tracer injections during one surgery in order to reduce the number of animals used for the study. After the last injection, the scalp incision will be closed, and the animal will recover. Oxymorphone will be provided postoperatively unless contraindicated by any neurological signs. Two days to two weeks following the procedures (depending on the specific tracers used), the macaque monkey will be heavily anesthetized with sodium pentobarbital and perfused with various aldehyde solutions. The brain will then be prepared in 30µm sections for analysis of neuronal projections to various sites in the brain.

Rhesus and fascicularis macaques have been chosen, as they provide the best model of human neuroanatomy. The number of animals listed is necessary because each set of injections traces a small population of neurons. Multiple independent injections are needed to define the functional circuits underlying memory and social behavior.

Fasting is only preliminary to surgery to prevent vomiting and aspiration.

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	MRI, BrdU and tracer injection*	12 immature Rhesus	3
2	MRI, BrdU and tracer injection*	12 mature Rhesus	3
3	MRI, BrdU and tracer injection*	12 Fascicularis	3
	*Choice of anesthetics at the discretion of the veterinarian from those listed below.		

## Categories of invasiveness

Category	Description
1	Little or no discomfort or stress <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

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

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

The ultimate goal of these studies is to understand the structure and function of the human brain. The macaque monkey provides the best available animal model for experimental analysis of primate neuroanatomy. The number of animals used is the minimal number needed to provide convincing and reliable descriptions of neuroanatomical patterns.

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

Building:

CRPRC

Room:

CRPRC Surgical Suite

Who will be the surgeon?

and various trained students.

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/Fascicularis	Ketamine	10-20 mg/kg	IM	During MRI, transport, and presurgery
"	Atropine	0.4mg/kg	SQ	During MRI, transport, and presurgery
"	Medetomidate	25-50µg/kg	IM	During MRI, transport, and presurgery
"	Xylazine	0.5-2 mg/kg	IM	During MRI, transport, and presurgery
"	Isoflurane	1-2.5%	inhalation	During surgery

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?

N/A

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

N/A

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

N/A

**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)

There will be postoperative pain associated with the craniotomy. Analgesics (oxymorphone or buprenex) will be provided as long as there are no postoperative contraindications (e.g. evidence of neurological dysfunction). Animals will be monitored daily for complications.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Anesthetics and analgesics are appropriate.

*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?

1/24/02

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
PubMed (NLM)	1966 - present	Hippocampus, amygdala, connections
Current Contents	1989 - present	Hippocampus, amygdala, connections
BIOSIS Previews	1985 - present	Hippocampus, amygdala, connections

What were your findings with respect to alternative methodologies?

There are no alternative strategies to determine brain connectivity.

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?

Death is not an endpoint. Animals will be sacrificed two weeks after the neural tracers have been injected in order to allow histological analysis of the brain.

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/ Fascicularis	Perfusion	Deep pentobarbital anesthesia	50-100 mg/kg	IV

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

All animals will be euthanized.



**Assurances for the Humane Care and Use of Vertebrate Animals:**

Principal Investigator's Statement:

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

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

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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**Committee Use Only Below**

<b>** Conditions necessary for Committee Approval:</b>
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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**ANIMAL ROOM SAFETY INFORMATION**

Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

**PROTOCOL # 9960****EXPIRES:**RUA#: 1409

BUA#: \_\_\_\_\_

CCA#: \_\_\_\_\_

Identity of Hazard:

3H-amino acids

Investigator Last Name:

First Name:

Email:

Department:

Phone:

Fax:

**Provide a short description of the agent:**

3H-amino acids are injected into the brain and incorporated into constituent proteins that are transported from cell body to terminals in labeled neurons.

This agent / material is hazardous for:  Humans only  Animals only  Humans and Animals  
For which Animal Species? \_\_\_\_\_

The agent can be spread by:  Blood  Feces/urine  
 Saliva/nasal droplets  Does not leave animal  
 Other: \_\_\_\_\_

**Describe any human health risk associated with this agent:**

Risk is associated with exposure to a radioactive substance. Total amount of material injected into the brain is 10 $\mu$ Ci. While above background counts have been observed in urine, levels provide minimal risk of exposure to human handlers. There is no evidence that this level of radioactivity is toxic to the injected animal.

**The precautions checked below apply to this experiment:**

- The researcher or his/her technicians are responsible for the feeding and care of these animals.  
 The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.  
 Cage  Stall  Water Bottle  Animal Carcasses  
 Bedding  Other: \_\_\_\_\_
- Cages must be autoclaved before cleaning.  
 Label cages and remove label after decontamination.  
 Animal carcasses must be labeled and disposed of as follows:  
 Incineration  Biohazardous Waste Container  
 Bag and Autoclave  EH&S will pick-up (2-1493).  
 All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:  
 Incineration  Biohazardous Waste Container  
 Bag and Autoclave  EH&S will pick-up (2-1493).

**Personal Protective Equipment Required:**

- The following personal protective equipment must be worn/used in the room:  
 Lab Coat/Coveralls  Shoe Covers/Booties  
 Disposable Gloves  Head Cover  
 NIOSH Certified Dust Mask  Disinfectant footbath  
 Eye Protection/Face Shield   
 Fitted Respirator Type: \_\_\_\_\_  
 Other: Describe: \_\_\_\_\_
- Personal protective equipment must be removed before leaving the room.  
 Personal protective equipment must be discarded or decontaminated at the end of the project  
 Hands, arms, and face must be thoroughly washed upon leaving the room  
 Full shower, including washing of hair, must be taken upon leaving the room.  
 Decontaminate Room (Inform ARS area supervisor when cage and/or room can be returned to general use).

**Provide any other information needed to safely work in this room:**