PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CNPRC

EH&S USE ONLY

PROTOCOL #_9985_

EXPIRES: _____

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

Species (common names):	Number:	Source:
Rhesus Macaque	12	CRPRC

Project Title: Transgene Therapy in Alzheimer's Disease

Overnight housing location:: CRPRC Day use only :

Animals will be maintained by: [X] Vivarium [ ] Investigator *(If investigator maintained, attach husbandry SOP's.)*

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

Animals will have an MRI to provide an individual map of their brain. Animals will then be trained on computer tasks to measure their cognitive function and attention span. These animals will then receive genetically modified fibroblast cells or a non-replicating viral vector to determine if their presence improves the animal's ability to focus attention and hopefully improve memory function. Drug challenges will be conducted during pre and post-operative testing to look at effects of the cholinergic system.

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.

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

Sick Animals	Dead Animals	Pest Control
[X] Call Investigator	[X] Call Investigator	[ ] Call Investigator
[X] Clinician to treat	[ ] Save for Investigator	[X] OK to use pesticides
[ ] Terminate	[ ] Bag for disposal	[ ] No Pesticides in animal area
[ ] Necropsy	[X] Necropsy	

Hazardous Materials *(only if in the animal room)*:

Infectious Agents?	[X] Yes [ ] No	Agent(s):	Adeno-Associated Viral Vector; lentiviral vector derived from HIV
Radioisotopes?	[ ] Yes [ ] No	Agent(s):	
Chemical Carcinogens?	[ ] Yes [ ] No	Agent(s):	
Toxic Chemicals?	[ ] Yes [ ] No	Agent(s):	

Is the project already funded?	[X] Yes [ ] No	Previously approved?	[X] Yes [ ] No
Proposed Funding Source:	NIH	Previous protocol number (if any):	8442

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

[ ]	Lab Animal Health Clinic (2-0514)	[X]	California Primate Research Center (2-0447)
[ ]	VMTH Large Animal Field Service (2-0292)	[ ]	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 intent of this study is to assess the use of somatic gene therapy utilizing a trophic factor in fibroblast cells or a lenti-NGF or AAV-NGF replication-incompetent viral vector to restore function in neurodegenerative diseases. The hypothesis is that fibroblast cells or a viral vector genetically modified to produce Nerve Growth Factor (NGF) can have a protective effect on cholinergic cell populations affected by neurodegenerative diseases. We will also assess the ability of cells or vectors cultured in Dr. Laboratory to facilitate cholinergic function. The significance of this project is two-fold. First, these experiments will help us understand mechanisms of neuronal plasticity and repair the adult primate brain, and secondly, they could have potential for therapy in the treatment of neurodegenerative disorders such as Alzheimer's Disease. Skin fibroblasts are collected from a rhesus and grown in cell culture. These cells are then transfected with a gene for NGF, or Beta galactosidase, a control gene. The animal is tested preoperatively with MRI and a variety of computerized cognitive and attentional tasks. A series of drug challenges to determine cholinergic system function using scopolamine (0.001-0.03mg/kg) IM and physostigmine (0.001-0.08mg/kg) IM will be conducted once prior to surgery and again post-surgery while testing on each computerized task. Following pre-surgical testing, animals receive intracranial grafts of suspensions of their own fibroblasts or the viral vector, genetically modified to produce NGF, or Beta galactosidase (B Gal) which is a reporter gene. In the third group, animals will be grafted with stem cells. Following surgery recovery of three to four weeks, they will begin post-op behavioral testing on the cognitive and attentional tasks. They will also have post-operative MRI's. Following completion of post-operative testing, animals will be deeply anesthetized, the brain exposed and a biopsy taken of cells grafts in the superficial cortex. These grafts will be utilized to determine gene expression. The animal will, immediately upon finishing the biopsy, be perfused with paraformaldehyde and euthanized.

b) Procedures employed in this project:

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

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

** 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 basic treatment to each animal will be quite similar to that found in the previous transgene studies. The table in section D will identify exactly what procedures will be involved for each animal. The generic procedures for each animal is as follows:

1. Animal is immobilized with Ketamine (10mg/kg) IM and a 2 cm diameter skin biopsy collected from the back. This biopsy is sent to UCSD for culture and transgene insertion. The biopsy is closed with 3-0 vicryl simple interrupted.
2. Animals are then behaviorally tested in the home cage utilizing a computer touch screen to measure cognitive function and attentional deficits. Animals must touch the computer in a certain way dependant on the task to receive a food treat/pellet. Animals on this testing regime will receive normal feeding in their home cage unless they are lacking motivation. If this is the case, the daily chow portion will be manipulated with notification to the veterinary staff. This would include decreasing the animal's daily chow. The animal will be weighed weekly in order to make sure that the animal is not losing too much weight. Once the performance on the computerized task is consistent, the normal chow portion will resume. Animals will be tested five days a week for 1-2 hours/day It is anticipated that it will take several months for each animal to complete testing.
3. Animals will be drug challenged during the pre-operative behavioral testing regime with scopolamine (0.001-0.03mg/kg) IM and physostigmine (0.001-0.08mg/kg) IM, both which have been shown to affect the function of the cholinergic system. The exact doses are to be determined on an individual animal basis. The doses will be given IM at a volume of 0.1ml/kg approximately 15-30 minutes prior to the testing session. The drug doses will be administered no more than 2 times per week. Three doses per compound will be evaluated, up to 4 times per dose. This will allow for setting a task rotation where it takes two different sessions to complete all of the tasks. Two observations will be taken at each dose for each task.
4. Animals will receive pre-operative MRI imaging. Animals are anesthetized with ketamine (10 mg/kg) IM and xylazine (1.5 mg/kg) IM or medetomidine (20-50 ug/kg) IM. Animals will then be placed in an MRI compatible stereotax and receive MRI imaging of the brain in a mobile unit at the CRPRC or at the UCD Imaging Center in Sacramento.
5. Animals will receive surgical grafting of modified fibroblast cells or viral vector to the brain. Animals will be anesthetized with ketamine (10 mg/kg) IM, given atropine (.04 mg/kg) IM, intubated, and placed on isoflurane. They will be placed in a stereotax and dorsal midline incision made. Upon exposure of the brain, 10 l of cell/vector suspension will be placed at each of five sites, a bolus of 10-50ul of

cell/vector suspension will be placed superficially into the caudal cortex and the dura closed, followed by replacement of the bone flap and skin closure. The B-Gal group is the control injection since those cells only express a reporter gene.

6. If the viral vector is injected at time of surgery, at 2 weeks and possibly 4 weeks post surgery, animals will be anesthetized with ketamine (10mg/kg) IM to have CSF samples taken to determine if there is anyvleakage of the viral vector. This will be repeated every 3 months until the end of the study.
7. Animals will then repeat procedures 2, 3 and 4 for post-op testing. The length of time for completion of the study is dependant on the individual animal's ability to complete the required cognitive tasks. This will vary for each animal but will continue until the animal is able to complete the testing regime, approximately 6-9 months.
8. Following completion of post-op testing, animals will be deeply anesthetized with ketamine (10mg/kg) IM, given atropine (1.5mg/kg) IM, intubated and placed on isoflurane. A craniotomy is then performed and a biopsy collected of cell grafts in caudal superficial cortex. The animal is then sedated under pentobarbital, perfused with paraformaldehyde and the brain collected for histology.

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, Behavior Testing, s + w NGF cells/vector	4	3
2	MRI, Behavior Testing, s + w B-Gal(control) cells/vector	4	3
3	MRI, Behavior Testing, s + w Stem cells/vector	4	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 the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

Rhesus monkeys have neuroanatomy and behavioral performance similar to that of humans. They also demonstrate changes in cognitive function and attention similar to that in humans with aging. A group size of 4 has been demonstrated in previous studies to provide significant results using a student's t-test.

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

Building: CRPRC Room: 1316

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 mg/kg	IM	For Biopsies, MRIs and prep for surgery
	xylazine	1.5 mg/kg	IM	MRI
	oxymorphone	1.5 mg/kg	IM	post operatively
	isoflurane	to effect	inhalation	during surgery
	Medetomidine	20-50 ug/kg	IM	MRI

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 a skin biopsy and neurosurgery. Oxymorphone will be routinely given following neurosurgery and may be provided after a biopsy. Any animal showing post surgical complications will be treated by the veterinary staff and euthanized if necessary. Complications could include stupor, head pressing and altered motor function. These complications are not anticipated.

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.

Oxymorphone 1.5 mg/kg IM for 2 days, euthanasia if post-op complications

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? January, 2002

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
Melvyl Medicine	1994-2001	trophic factors, NGF, viral vectors, monkey
Neuroscience Abstract	1996, 1997, 1998, 2001	review of abstracts on NGF

What were your findings with respect to alternative methodologies?

Numerous studies have been conducted on neurotransplantation and use of genetically altered cells and viral vectors in rodents and nonhuman primates demonstrating anatomical effects. There have not been studies to demonstrate the behavioral effects of neurotransplant on cognitive function and attentional behavior.

Has this study been previously conducted? [ ] Yes [X] 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 all be euthanized at the end of the study as described.

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	Drug Overdose	Pentobarb	60 mg/kg followed by perfusion	IV

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

Any animals not operated on will be returned to the colony.

n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don't need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

Last Name	First Name	Middle Name	UC ID Number or SSN	Email Address

Occupational Health Program:

Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at ***http://clueless.ucdavis.edu/health/*** or read the UC Davis Policy & Procedure Manual 290-25.

Training:

Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at ***http://clueless.ucdavis.edu/.***

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.

Principal Investigator	*Rank / Title*	*Date*

CRPRC Director	*Date*

Committee Use Only Below

** Conditions necessary for Committee Approval:

Final Disposition of this protocol:

_____ Approved

_____ Not Approved

_____ 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.

Campus Veterinarian	*Date*

ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL #__9985__
EXPIRES: _____

RUA#: _____ BUA#: 0462 CCA#: _____

Identity of Hazard: non-replicating lentiviral vectors or adeno-associated viral vectors

Investigator Last Name:		Department:	
First Name:		Phone:	
Email:		Fax:	

Provide a short description of the agent:

Retroviral and AAV-based vectors are the most common vector systems used for gene therapy. The vectors are replication-defective and the only potential infection risk is if recombination occurs between vectors of the packaging sequences, which could lead to emergence of replication-competent viruses (not likely). Studies in rats have demonstrated that the replication deficient vector is cleared within 48 hours.

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 [X] Does not leave animal
[ ] Other:

Describe any human health risk associated with this agent:

Vectors have all viral genes removed and thus are replication-defective. AAV is nonpathogenic in humans. There are no known cases of accidental human infection or recombination, to date. Previous studies in rats have failed to detect any replicant competent vector.

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.
[X] Animal carcasses must be labeled and disposed of as follows:
- [X] Incineration (BFI pick up) [ ] 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:

[X] The following personal protective equipment must be worn/used in the room:
- [X] Lab Coat/Coveralls [ ] Shoe Covers/Booties
- [ ] Disposable Gloves [ ] Head Cover
- [ ] NIOSH Certified Dust Mask [ ] Disinfectant footbath
- [X] 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: