

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

EH&S USE ONLY

PROTOCOL # 9953**EXPIRES:**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus macaques	16	CRPRC

Project Title	Role of the Amygdala in Processing Social Cues		
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.

Adult monkeys will be implanted with an eyecoil, skull-mounted recording electrodes, intracranial recording electrodes and infusion cannulae. Inactivating agents will be infused into the amygdala and behavioral and cognitive testing will be conducted. Procedures include blood sampling, MRI and histology. Medical procedures may require food restricting animals.

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.

Individually housed at the CRPRC.

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 checked="" 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 type="checkbox"/> Necropsy	

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

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	Adeno-associated virus: BUA pending
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:	McArthur, McDonald	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> 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:		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 pcstillman@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 brain area known as the amygdala has been strongly implicated in mediating social signals. It is known in Rhesus monkeys that visual information enters the amygdala via its lateral nucleus. We intend to test the hypotheses that 1) the amygdala is required for normal processing of social and emotionally provocative visual cues (e.g., facial expressions) 2) the amygdala is required for normal social interaction in monkeys, and 3) the amygdala generates asymmetrical EEG signals in the frontal cortex. To accomplish this, we propose two sets of experiments.

First, 4 adult male and 4 adult female Rhesus monkeys will be trained on 2 tasks. First, they will be trained to focus on a specified point of light presented from a computer screen, while passively viewing pictures of varying emotional quality (e.g., landscape, animals, monkey faces) also presented from a computer screen. Second, they will be trained to recognize conspecific facial expressions using pictures and touchscreen response technology. Recordings of their brainwaves will be done using electrodes implanted in their skull and intracranially in their amygdalas, facial response areas, and frontal brain regions involved in emotional processing. Once normal brainwave responses are recorded, the amygdala will be reversibly inactivated using muscimol, a pharmacological agent, that acts as a GABA-ergic agonist which inhibits neuronal activity (, et al., 2002; et al., 2001; , 2001; , 2001), in the amygdala,. We will measure the effect of reversible inactivations of the basolateral amygdala on the behavioral and neural measures of social information processing. Note: inactivation is temporary and will only last about one hour, at which point the muscimol is metabolized by local brain enzymes and no long-term effects on neuronal activation in the amygdala are expected. Temporary inactivation will allow us to use each monkey as his/her own control, thereby reducing the number of animals needed for this research.

Second, 4 adult male and 4 adult female Rhesus monkeys will be observed in dyadic social interactions and their behavior will be quantified using standard measures and an advanced ethogram. They will also be trained to focus on a specified point of light presented from a computer screen, while passively viewing pictures of varying emotional quality (e.g., landscape, animals, monkey faces) also presented from a computer screen. In addition, they will be trained to recognize conspecific facial expressions using pictures and touchscreen response technology. As described above, recordings of their brainwaves will be done using electrodes implanted in their skull and intracranially in their amygdalas, facial response areas, and frontal brain regions involved in emotional processing. Once normal brainwave responses are recorded, an adeno-associated virus carrying a gene for a specific bacterial protein that forms a hormone receptor will

then be infused into the amygdala of the monkeys during a neurosurgical procedure. This gene will be incorporated into the nuclei of amygdala neurons and only those neurons that carry the gene (i.e., transfected neurons) will produce the bacterial receptor for which there is no endogenous ligand in the mammalian brain. This protein will insert into the cell membranes of the transfected neurons but will have no effect on the amygdala or on behavior until a bacterial hormone is infused into the transfected brain area. Once this happens, the bacterial protein will inactivate transfected neurons for several hours, but neuronal activity will eventually return to normal. During this period of inactivation, neuronal measures such as visual response testing will be recorded as specified above. Further, the persistence of inactivation in this set of experiments will allow us to test our second hypothesis by measuring behavior in freely moving subjects within dyadic social interactions.

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 checked="" 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 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.)

Reversible Inactivation Using Muscimol

Subjects and training. Subjects will be mother-reared adult male (n=4) and female (n=4) rhesus monkeys (aged 3-6 yrs). They will be housed individually and given free access to food and water in their home cages. They will be trained to sit in a primate chair while performing 2 visual discrimination tasks using a touchscreen computer: 1) conspecific face vs. nonfacial stimuli and 2) conspecific facial expression vs. conspecific facial expression (eg. Fear grimace vs. threat). Once subjects attain $\geq 80\%$ correct on all discrimination tasks, they will undergo surgery (see below) and retraining on the above discrimination tasks. They will also be trained to sit in a primate chair while visually fixating on a specified point of light and passively viewing images presented on the same computer screen, until they attain $\geq 90\%$ fixation. All training will use operant conditioning with food reward.

Stimuli and procedure. Stimuli will be compressed TIFF (Tagged Image File Format) images presented on a computer screen against a black background. Non-conspecific images will be obtained from established image databases and will be categorized as emotionally evocative (e.g., image of a snake) or not (e.g., image of a landscape). Images of conspecific facial expressions will be obtained via video capture using full color, high resolution videotapes of induced facial expressions in single monkeys (e.g., threat, fear grimace, lip smack, neutral).

Subjects will be tested on their ability to discriminate between facial and nonfacial stimuli and between two different facial expressions (e.g., threat vs. lip-smack) in a two-alternative forced-choice task. While chaired, subjects will be presented with two images (e.g., a face and a landscape), one of which is the correct choice. Subjects will touch the image on the screen to register their choice and to begin the next trial. Correct choices will be validated by food reward (sucrose pellets) delivered by an affixed pellet dispenser. Incorrect choices will be indicated by lack

of reward and by a 3-second timeout during which a black screen is presented. Subjects will be able to move their eyes freely across the screen.

Following surgery, the subject's head will be restrained during testing and eye movements will be monitored using a search coil surgically implanted around one eye. A field coil surrounding the subject's head will create a weak magnetic field that will induce a measurable current in the eye coil each time the subject's eyes move.

Subjects will also be trained to visually fixate a spot of light presented at the center of an image, shown against a black background. The subject's head will be restrained and eye movements will be monitored, as specified above. Fixation will be defined as maintaining visual focus within a defined box ($1^\circ \times 1^\circ$ of visual angle) for 4 seconds. Correct fixation will be indicated with food reward while failure to fixate will be indicated by lack of reward and a 3-second black-screen time-out before going on to the next trial.

Surgery. Following adequate performance on the discrimination tasks, subjects will undergo magnetic resonance imaging (MRI) to determine exact individual coordinates for surgical implantation. Subjects will be awake when transported from CRPRC to Sacramento for MRI analysis and monitored by a trained technician during the move. Once at the imaging center the attending vet or AHT will anesthetize the animal with ketamine (10mg/kg), and Metatomadine (20mg/kg) and determine if, for the welfare of the animal, a catheter and tracheal tube are medically necessary. Animals will be given Atropine (.04mg/kg) sub-cutaneously and placed in a MRI compatible stereotaxic apparatus for imaging. Following MRI, animals will be returned to CRPRC. The animals will be monitored by a trained technician during the return trip to CPRC.

Surgery will proceed a few days thereafter, for implantation of one eye coil; two (bilateral) MRI-compatible infusion cannulae, one in each amygdala; approximately 6 low-impedance and 6 high-impedance recording electrodes (tungsten), bilaterally placed in the amygdala (4 electrodes), the face-responsive region of the superior temporal sulcus (4 electrodes), and area 13 in the orbitofrontal cortex (4 electrodes); 11 MRI-compatible low-impedance electrodes (FZ, F3, F4, FT7, FT8, CZ, C3, C4, PZ, P3, P4) implanted into the skull in a standard electroencephalographic (EEG) configuration; and 3 pins implanted into the skull for head restraint. General procedures will conform to surgical SOP.

Briefly, a midline incision will be made and bilateral craniotomies performed directly above the amygdala, the facial responsive region of STS and area 13 of orbitofrontal cortex. Electrophysiological recordings will also be performed to confirm and further define the exact coordinates for the intracranial cannula and electrode implantation. Cannulae and intracranial electrodes will be free to move within the diameter of short guide cannulae cemented to the skull using dental cement. Head restraint pins will be fixed to the skull with screws and dental cement.

An eye coil will be implanted following standard procedures outlined by et al., (1980). The cornea will be separated from the conjunctiva and a recess will be created by blunt dissection between the conjunctiva and the sclerotica. The eye coil, made from Teflon-insulated fine stainless steel wire, will then be placed into this recess. The conjunctiva will be sutured and the coil attached with golden pins to a small connector cemented to the skull. Insulated wires from the electrodes and eye coil will be glued to the skull and fed through the midline incision near the front of the head. The incision will be sutured leaving a small opening for the wires to pass through. This procedure takes 20-25 minutes, and is usually followed by moderate pain and swelling of the conjunctiva. Immediately after surgery respiration and cognitive status will be monitored by a veterinarian until the vet staff has declared the subject fit to return to the home cage. Subjects will be monitored regularly until demonstrating a full recovery and good eating habits.

Electrophysiology. Electrophysiological recordings from low- and high-impedance electrodes and from skull-mounted electrodes will be made while

subjects execute the discrimination tasks and the fixation task. Electrodes will be used for recording only, not for stimulation. Equipment will be properly grounded to ensure that subjects do not receive electrical stimulation during recording.

Infusions. Once all behavioral and electrophysiological measures have been established in untreated animals, infusions will begin. After the subject is chaired and head-restrained, a few minutes prior to testing, uni- or bilateral injections of 700 nanoliters (nL) of either saline or muscimol (0.1% in saline) will be made via amygdala infusion cannulae using a Hamilton syringe with a tip extending 1 mm beyond the end of the cannulae. A trained AHT from Centralized Services at CPRC will be present to monitor and assist with the infusions and make certain that subjects are not adversely affected.

Reversible Inactivation Using Gene Transfer

Subjects and training. Subjects will be mother-reared adult male (n=4) and female (n=4) monkeys (aged 3-6 yrs). They will be housed individually and given free access to food and water in their home cages. They will be trained as specified above.

Stimuli and procedures. These are identical to those stated above. Additionally, subjects will be assessed using social testing in dyadic interactions. Dyad observations (observing the interactions of two novel or familiar monkeys) will take place between familiar (from the same cohort) and unfamiliar (from different cohorts) pairs in the large (18'X 7'), open arena, social enclosure. Each subject will be observed after receiving infusion of either saline or bacterial protein; thus each subject will serve in control and experimental conditions. Subjects will be boxed and delivered from their home cages to the testing area and loaded into temporary housing cages. Two animals (one familiar or novel treated and one familiar or novel untreated), will then be boxed and loaded into one of two stimulus release cages affixed to the large social arena. The opaque and grill doors will then be raised releasing both animals into the social arena at which point they will be allowed to interact freely with one another. Trials will be thirty minutes in duration, animals will be under continuous observation by two trained behavioral observers who will record each animals behavior and location using an advanced ethogram and the Windows 2000 Observer program.

Surgery. As above.

Electrophysiology. As above.

Gene transfer. We will use the adeno-associated virus AAV to deliver the gene for the drosophila allatostatin receptor (AlstR). This receptor, *when activated by its ligand (allatostatin)*, hyperpolarizes neurons and effectively silences them for several hours. Incorporation of cell type specific promoters into the virus will allow specific targeting of selected amygdala cell types. Injection of the replication incompetent virus into the brain: Animals are initially anesthetized with ketamine (IM). An IV line is inserted and the trachea intubated. They are then placed in a stereotaxic holder and anesthesia is switched to inhaled isoflurane in oxygen. A midline scalp incision will be made and a craniotomy drilled through the skull to expose the brain region of interest. A small cut will be made in the dura and a glass pipette filled with the replication incompetent, recombinant AAV will be lowered into the brain. The replication incompetent virus will be injected into the brain either by pressure or iontophoretically. The AAV injections will be a volume of 2 microliters dissolved in 0.1M phosphate buffered saline and 1-8 injections will be made into the amygdala depending on the area that will be inactivated. The pipette will then be removed and the scalp sutured shut. Anesthesia will be discontinued and the animal monitored closely until fully recovered.

Infusions. As above, except that infusions will involve saline or bacterial protein. The drosophila allatostatin receptor (AlstR) will be activated in those animals that receive the infusion of bacterial protein. Specific cells within the amygdala will be hyperpolarized causing them to be temporarily silenced. Initial infusions prior to social dyad testing will be done under the guidance and assistance of an AHT from Centralized Services at CPRC.

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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, muscimol inactivation of amygdala	8	3
2	Adult, gene transfer inactivation of amygdala	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?

Facial expressions are known to be a significant cue of social interaction in Rhesus monkeys, while it is not thought to be important in rodents, cats or ferrets. Additionally, Rhesus monkeys have consistently been used as nonhuman animal models in the study of socioemotional behavior in humans. The animals included in these treatment groups are the smallest numbers that can be used to assure the prospect of a statistically significant finding. Behavioral measures

will be subjected to parametric and nonparametric analyses, including ANOVA, t-test, and the Mann-Witney.

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 HCl	7-10	IM	Pre-surgery
Rhesus	Isoflurane	To effect	Inhalation	During surgery, up to 12 hours
Rhesus	Fentanyl	7-10mcg/kg/hr	I.V. Infusion	During surgery, up to 12 hours
Rhesus	Metatomadine	30mcg/kg	IM	Pre-surgery
Rhesus	Atipamezol	15mg/kg	SC	May be used post-surgery to reverse effects of Metatomadine
Rhesus	Atropine	.04mg/kg	IM	Pre-surgery
Rhesus	Buprenorphine	.01-.03mg/kg	IM	Daily for 2-3 days following surgery for post-surgical pain management.
Rhesus	Muscimol	700 nanoliters (nL)	Intra-cranial	Prior to outlined behavioral and visual response testing

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)

Within the last five years, the P.I. has performed 7 one-stage bilateral amygdalectomies, 17 two-stage bilateral amygdalectomies/hippocampalectomies in adult Rhesus monkeys and 16 amygdalectomies/hippocampalectomies in infant Rhesus monkeys using approved experimental protocols at the CRPRC. Animals with amygdala or hippocampal lesions continue to eat and drink adequately to maintain body weight. The reversible inactivations described here should be much less stressful, since they will not result in complete and permanent damage to the entire amygdala. Some stress may be experienced by subjects during the first two weeks following surgery, during which time, intense post-operative care will be provided as necessary by the CRPRC staff. Post-operative complications to the subjects may include, but are not limited to, inanition, dehydration, failure to thrive, and infection primary or secondary due to handling.

Since the procedure causes temporary damage to brain circuits, we expect temporary changes in behavior including changes in emotional responses. Animals in the social condition might experience some stress during dyadic interactions and will be monitored by a trained observer throughout social testing.

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.

Veterinary staff at the CRPRC will be directed to provide Buprenex as necessary for the post-surgical relief of pain.

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?

10/15/01 & 1/5/02

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
Medline	1966-present	Amygdala, muscimol, social behavior, Rhesus monkey, primate, gene transfer
PsychInfo	1966-present	Amygdala, muscimol, social behavior, Rhesus monkey, primate, gene transfer
PubMed	1990-Present	Muscimol, Inactivation, neurons

What were your findings with respect to alternative methodologies?

The proposed methodology produces the most precise temporary inactivation of the amygdala. The behavioral studies that have been proposed are unique and provide for the most sophisticated analyses of behavioral consequences of amygdala inactivations to date. There are no better methodologies available for the proposed studies.

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.

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k) **Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

Euthanasia may be necessary to determine the precise location of the implanted cannulae and electrodes. However, every attempt will first be made to use magnetic resonance imaging and positron emission tomography for localization. If this is successful, animals will be euthanized only after the completion of behavioral testing.

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	Sedation	Ketamine HCL	10mg/kg	IM
Rhesus	Overdose	Sodium pentobarbital	60mg/kg	IV

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

Animals not euthanized will continue to be utilized in future studies combining reversible inactivation, behavior and electrophysiology.
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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# 9953**EXPIRES:** _____

RUA#: _____

BUA#: Pending

CCA#: _____

Identity of Hazard: Adeno-associated virus (AAV)

Investigator Last Name: _____ Department: _____

First Name: _____ Phone: _____

Email: _____ Fax: _____

Provide a short description of the agent:

Adeno-associated virus (AAV) is commonly used as a viral vector for gene transfer. This viral vector is replication incompetent. The risk of recombination to a pathogenic form is highly unlikely. AAV is not pathogenic in humans.

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

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:

All viral vectors are replication defective and non-pathogenic. To date, there are no known cases of accidental human infection. Furthermore, this research has proposed that the virus be injected directly into the brain region targeted to receive the novel gene. Once injected the AAV cannot pass the blood/brain barrier and will be broken down into harmless derivatives by natural enzymes within the brain.

The precautions checked below apply to this experiment: *Standard CRPRC handling and housing applies.

- 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: None
 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:

