

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

EH&amp;S USE ONLY

**PROTOCOL #10141  
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	8	CRPRC

<b>Project Title</b>	Role of Amygdaloid Nuclei in Responses to Facial Expressions		
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 a headpost for head constraint; chronic, skull-mounted recording electrodes; chronic intracranial recording electrodes; and chronic recording/infusion wells over the amygdala. Infusion cannulae will be introduced into selected regions of the amygdala. Inactivating agents will be infused into the amygdala and behavioral and cognitive testing will be conducted. Cognitive testing will require water control. 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.

Housed at the CRPRC. Pair housing preferred. Subjects will be maintained on the AUCAAC-approved water control regimen.

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 type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	McDonnell Foundations	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](mailto: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 emotionally provocative social information. Lesions to the amygdala in humans result in deficits in recognizing facial expressions of emotion. Functional neuroimaging of the amygdala in people with autism, depression and schizophrenia shows abnormal activity compared with controls. People with depression also demonstrate an abnormal frontal asymmetry in resting EEG signals and in EEG signals elicited by emotionally provocative images.

The Rhesus monkey is a powerful animal model of human social communication. Anatomical studies in Rhesus monkeys show that visual information enters the amygdala primarily via its dorsolateral and basal nuclei. 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) and 2) the amygdala generates asymmetrical EEG signals in the frontal cortex elicited by emotionally provocative images. To accomplish this, we propose the following experiment.

Four adult male and four adult female Rhesus monkeys will be trained to sit in a primate chair in front of a computer monitor and perform visual image discriminations and image category discriminations. They will respond by **R**eleasing a handle to some images (R images) and by **H**olding the handle to other images (H images). They will undergo chronic implants of a headpost for head constraint and of skull-mounted electrodes for resting electroencephalograms (EEGs, brainwaves) measuring event-related potentials (ERPs; these are brainwaves time-locked to certain stimulus and behavioral events). All chronic implants will be MRI-compatible. They will then be required to make discriminations while visually focusing on a small point in the middle of the computer monitor (i.e., visual fixation). Eye movements will be monitored using a high-speed infrared camera system. Eye coils will not be required. Subjects will acquire the ability to make the following category discriminations: 1) neutral face vs. fear grimace; 2) neutral face vs. full threat; 3) neutral face vs. non-facial object. Their brainwaves will be measured during this behavior. Following this, they will undergo bilateral chronic implants of recording/infusion wells that are large enough to cover the dorsolateral and basal amygdaloid nuclei. The implants will be guided by structural magnetic resonance imaging (MRI). After reacquiring normal discrimination, visually active areas of the amygdala will be identified and characterized using acutely placed electrodes in the amygdala, inserted via the implanted wells. Both neurophysiological responses and followup structural MRIs will be used to confirm entry into the correct amygdaloid nuclei. Following this, muscimol, a drug that inhibits neuronal activity by acting via fast neuronal ion channels known as GABA receptors, will be infused into the

amygdala through acutely placed cannulae, again via the implanted wells. If muscimol is ineffective alone, other compounds, functionally similar to muscimol in acting at GABA receptors, will be used. They will be used alone or as a cocktail. We will measure the effect of transient inactivations of the visually active amygdaloid nuclei on the behavioral, EEG and ERP measures of processing emotionally provocative social information, compared to behavior, EEG and ERP during infusion of artificial cerebrospinal fluid. Note: inactivation is temporary and will last from 1.5-7 hours. Muscimol and other similar compounds are broken down locally in the brain and no long-term effects on neuronal activity are expected. These agents have effects similar to commonly used anti-epileptic medications. Transient inactivation will allow us to use each monkey as his/her own control, thereby reducing the number of animals needed for this research.

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

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

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

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

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

#### **Transient Intracranial Inactivation**

**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 pair-housed if possible and given free access to food in their home cages. They will be placed on the AUCAAC-approved water control regimen. They will be trained to sit in a cognitive/behavioral testing primate chair in front of a computer monitor while performing visual image and category discrimination tasks. They will use a response handle and receive liquid reward (e.g., Tang or fruit juice). Category discriminations include: 1) face vs. nonfacial objects and 2) one facial expression vs. another facial expression (e.g., fear grimace vs. neutral, threat vs. neutral). Once subjects attain  $\geq 80\%$  correct on image discrimination tasks, they will undergo surgery (see below) and retraining on these tasks. They will then be trained to visually fixate a spot on the computer monitor (2 degrees of visual angle on a side) while performing the image discrimination tasks. Following this, subjects will be trained to make category discriminations while visually fixating, after which they will undergo a second surgery (see below) that will be guided by individual structural magnetic resonance imaging (MRI). Subjects will be retrained on all visual fixation/discrimination tasks. All training will use operant conditioning with water control and liquid reward.

**Stimuli and procedure.** Stimuli will be images presented on a computer screen against a background. Non-facial images will be obtained from established image databases and will be categorized as emotionally provocative (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., full threat, mild threat, yawn, fear grimace, lip smack, neutral). Acquisition of video images have been covered by previous IACUC protocols.

Subjects will be trained in order to perform the following tasks: a) receiving liquid reward from a sipper tube; b) holding grasp on a response

handle; c) holding grasp on the presentation of a colored square (e.g., green), releasing grasp on the presentation of a differently colored square (e.g., red); d) holding/releasing grasp on the presentation of non-facial images randomly labeled H/R, respectively. Subjects will then undergo the first of two surgeries (see below). Following surgery, the subject's head will be restrained during retraining on the discrimination tasks. Visual fixation training will also begin, and subjects will be required to visually fixate a point on the computer monitor not exceeding 2 degrees of visual angle on a side. Eye movements will be monitored using a high-speed infrared camera. Surgically implanted search coils will not be used.

Subjects will be required to maintain visual fixation while performing discrimination tasks at a level of  $\geq 80\%$  correct. Correct trials will be rewarded with juice paired with a tone. Incorrect trials will be indicated by a darkening of the screen for 2 seconds - 5 seconds along with a low-pitch tone.

Once subjects are at criterion for R/H image discrimination, category discrimination training will begin. Once these tasks are acquired at  $\geq 80\%$  correct, subjects will undergo a structural MRI and a second surgery (see below). After retraining on all visual fixation/discrimination tasks, transient inactivation studies will begin (see below). The effects of transient inactivation will be measured by the following behaviors: a) percent correct in the discrimination task, b) reaction time in the discrimination task and c) pupillary dilation response (measured non-invasively using the infrared camera).

**Surgery and MRI. Surgery I.** Following adequate performance on the image discrimination tasks, subjects will undergo surgery to implant a) 1 MRI-compatible headpost into the skull; and b) 11 MRI-compatible low-impedance electrodes into the skull in a standard electroencephalographic (EEG) configuration (electrode positions will correspond to FZ, F3, F4, FT7, FT8, CZ, C3, C4, PZ, P3, P4). General procedures will conform to SOP.

Briefly, a midline incision will be made and skull electrodes will be implanted, followed by the headpost. Dental cement will be used to reinforce the implants. The headpost will be held to the skull via MRI-compatible screws implanted into the skull. All implants will be done to avoid problems accessing the area required by Surgery II. 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.

**Surgery II.** Following adequate performance on the image and category discrimination with visual fixation, 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, an i.v. catheter and tracheal tube are medically necessary. Animals will be given Atropine (.04mg/kg) subcutaneously 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.

At least 1 week later, subjects will undergo surgery to implant a) bilateral MRI-compatible recording/infusion wells over the dorsolateral and basal amygdaloid nuclei; and b) 2 low-impedance MRI-compatible depth electrodes, bilaterally into the orbitofrontal cortex. General procedures will conform to SOP. Briefly, a midline incision will be made and bilateral craniotomies will be performed directly above the amygdala and above the orbitofrontal cortex. Electrophysiological recordings will also be performed to confirm and further define the exact coordinates for electrode implantation.

**Electrophysiology.** Electrophysiological recordings from low- and high-impedance electrodes and from skull-mounted electrodes will be made while subjects execute the discrimination tasks while holding visual fixation.

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.

**Targeting Assessment.** In order to verify that the correct amygdaloid nuclei have been targeted for infusion, at least 1 MRI assessment will be done for each subject. Bilateral MRI-compatible electrodes will be lowered into putative amygdaloid areas that are visually responsive and selective. Once electrodes have been lowered into these areas, they will be temporarily cemented in place inside the wells. No more than 1 day later, subjects will undergo an MRI, following procedures detailed above, to determine if the electrodes were in fact placed into the amygdala. This assessment will be carried out, with intervals defined by SOP, until targeting of the correct amygdaloid nuclei is verified. The extent of neural inactivation resulting from the infusions will be assessed using simultaneous neurophysiological recordings at infusion sites and sites nearby.

**Infusions.** Once all behavioral and electrophysiological measures have been established in untreated animals and targeting has been verified, infusions will begin. After the subject is chaired and head-restrained, behavioral testing will begin. Combination electrodes and cannulae will be lowered into the dorsolateral and basal amygdaloid nuclei. Once visually responsive neuronal activity is acquired at the appropriate depth, uni- or bilateral infusions of 0.5 microliters ( $\mu\text{L}$ ) - 1  $\mu\text{L}$  of artificial cerebrospinal fluid (i.e., physiological saline), followed by behavioral and electrophysiological testing. Then infusions of muscimol (or other GABAergic agonists) (0.5 - 1  $\mu\text{L}$ ; 1 micrograms ( $\mu\text{g}$ )/ $\mu\text{L}$  - 4  $\mu\text{g}/\mu\text{L}$  in saline) will be made, again followed by behavioral and electrophysiological testing. Neurophysiological recording at infusion sites and at sites nearby will verify local effects of infusions.

**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 (or another GABAergic agonist) inactivation of amygdala	8	3

## 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?

Facial expressions are known to be a significant cue of social interaction in Rhesus monkeys, while they are 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-Whitney.

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

Rhesus	Muscimol (or other GABAergic agonists)	1 $\mu$ L @ 0.5 $\mu$ g - 4 $\mu$ g	Intra-cranial	post-surgical pain management. Prior to outlined behavioral and visual response testing
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**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 19 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 inactivation of brain circuits, we expect temporary changes in behavior including changes in emotional responses.

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 [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?

10/15/01 &  
5/20/02

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
PsychInfo	1966-present	Amygdala, muscimol, social behavior, Rhesus monkey, primate
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.

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. In any event, animals will be euthanized only after the completion of extensive 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?

At the end of extensive behavioral testing, all subject animals will be euthanized to precisely determine the location of electrodes and patency of the amygdala.





6/4/03

Pre review questions

Hi,

I have received and pre reviewed the recently submitted amendment to protocol 10141. I just have a few questions to clarify a few points. I have attached a copy of the amendment for ease of making revisions.

Thanks in advance,

Amendment to Protocol 10141

1. In section a. 1) you use the term "A-weighted". Please clarify what you mean when you are using this term.

X-POP3-Rcpt:  
Date: Wed, 4 Jun 2003 16:16:41 -0700 (PDT)  
From:  
X-X-Sender:  
To:  
cc:  
Subject: Re: Fwd: pre review questions amendment to protocol 10141

Dear \_\_\_\_\_,  
Thanks for those questions. I've revised the proposed amendment and I've put the responses in bold type, so they're easy to find.  
Let me know if there are any other questions.

**Date:** Revised June 4, 2003  
**To:** Animal Care and Use Administrative Advisory Committee  
 c/o Office of the Campus Veterinarian  
**From:** , Principal Investigator  
**RE:** Amendment to Protocol #10141

This describes an amendment to Protocol 10141 that asks for permission to 1) display dynamic visual and/or auditory stimuli (i.e., movies, vocalizations, various sounds, etc.) to the subjects already approved for use under this protocol; 2) use a human intruder paradigm with the subjects already approved for use under this protocol; and 3) use a fear conditioning paradigm with the subjects already approved for use under this protocol.

a. Proposed Changes

No new animal subjects are requested under this amendment.

No new personnel additions to the protocol are requested at this time.

The experimental procedures we are requesting permission to perform are given as follows:

**1) Display of dynamic visual and/or auditory stimuli**

We want to learn the role that the amygdala plays in learning, recognizing and responding to environmental stimuli, particularly affectively negative social stimuli. Currently, we are approved to use static visual stimuli of Rhesus faces and other objects to induce cognitive/affective states. We are asking permission to also display silent movies, audible movies and/or just sounds. The dynamic visual stimuli would include movies of other Rhesus monkeys performing threatening, neutral or affiliative/submissive behavior, movies of snakes and reptiles, and movies of humans showing direct stare, etc. Sounds would include Rhesus monkey vocalizations, human voices, sounds from the environment, white noise, tone pips, etc. We ask for permission to present these sounds either with accompanying visual stimuli or without, at sound pressure levels not to exceed 90 decibels (dB), A-weighted. **A-weighted means that the sound level meter will be set to compensate for the fact that humans (and monkeys) don't perceive sounds at high frequencies as loudly as sounds at lower frequencies. So, the A-weighting provides a better measure of what the monkey actually hears than not weighting the input by frequency.** We do not anticipate presenting these sounds at sustained levels close to 90 dB, but some threat vocalizations and coos may be this loud intermittently.

We plan to display visual stimuli over a computer monitor or television, and sounds using standard speakers or headphones, while our subjects have their heads fixed and are sitting in the primate chair.

**2) Use of a human intruder paradigm**

We plan to use a variation of a human intruder paradigm already in use by \_\_\_\_\_ and our lab here at the California National Primate Research Center. While the subject is sitting with his head fixed and in the primate chair, a human will be positioned in front of the subject in one of four conditions. The human will either stand or kneel so that her/his face is no less than 27 inches away from the subject and will either face the subject, making direct eye contact, or present his/her profile to the subject. The human will not do anything else that suggests a threat or harm to the subject. Each condition will last for no longer than 6 minutes.

A CNPRC staff research associate or veterinarian will be present during the experiment, to call off the experiment if the subject struggles against his headpost in response to the human intruder.

**3) Use of a fear conditioning paradigm**

We plan to use fear conditioning to induce an affectively negative state in our subjects, and thus drive the EEG asymmetry known to be associated with such states.

Fear conditioning relies on making an association between an innocuous stimulus, like a light or a low-intensity sound, and a stimulus that is mildly aversive, like an air puff delivered in close proximity to the face. After a subject has learned an association between these 2 types of stimuli, using only the light or the sound induces a state of conditioned

fear. In conditioned fear, the simple presentation of the light or sound evokes the same reactions in the subject as does the mildly aversive stimulus.

We plan to condition the subjects to associate a light and/or sound (i.e., the conditioned stimulus) with an air puff (unconditioned stimulus) delivered in proximity to a subject's face. We will then use the conditioned stimulus to induce a state of conditioned fear, and thus drive the EEG asymmetry we hope to see. We will measure the amount of conditioning by using an accelerometer sitting underneath the subject's restraint box, to measure the change in the subject's body movement due to the air puff and light/sound.

To form the association between the conditioned stimulus with the unconditioned stimulus, subjects will be individually placed in a custom-built wooden chamber (70 cm X 48 cm X 109 cm) that contains a plexiglass restraint box. **The wood of the chamber was sealed with a water-resistant paint. The subjects do NOT come into contact with the sealed walls of the chamber, since they are always confined within the plexiglass restraint box. The chamber will be washed and disinfected using water, bleach solution and Cavicide. Disinfectants will be washed away with water prior to the subject being placed into the chamber, to reduce the risk that the subject inhales disinfectant vapors.** The plexiglass box is designed to hold monkeys weighing between 10 kg and 17 kg. The wooden chamber is sound-attenuated and is ventilated using an electric fan producing 65 decibels (dB) of noise in the chamber. The wooden chamber will be equipped with a speaker for playing sounds and with different colored lights. An air compressor will sit outside of the chamber and a tube will extend from the compressor to the box, to an outlet near the subject's face. Sounds and/or lights will be presented to a subject preceding the presentation of an air puff to the subject's face. We will determine the interval that gives the best conditioning. This setup is the same as described under a recent amendment (4/7/03) to Protocol 10187.

After conditioning, the subject will be tested in the booth currently approved for use under Protocol 10141. We will measure EEG in response to the presentation of the conditioned stimulus (i.e., light or sound).

#### b. Justification

The goals of this research program demand that we see frontal EEG asymmetry associated with stimuli thought to be affectively negative. If we cannot induce this asymmetry, we have no metric to probe for the amygdala's role in generating the asymmetry. It is known that Rhesus monkeys do exhibit this EEG asymmetry under the conditions of hand restraint by a human handler. We want to avoid this invasive procedure, and maintain more control on the stimulation we use to induce the anticipated EEG asymmetry.

We have completed the experiment with static visual stimuli and have analyzed the data for 1 subject. We were expecting to see large EEG asymmetries over the frontal lobes while the subject looked at images of conspecific facial threats, compared to facial neutrals. We didn't see this, and we believe it is because the static stimuli are not the most effective way to simulate the real threat behavior of a conspecific. So, we are asking permission induce the EEG asymmetry in 3 other ways, described above. The justifications are given here:

#### 1) Display of dynamic visual and/or auditory stimuli

These stimuli are more naturalistic than static stimuli, in that they involve natural movements of animals, especially other Rhesus monkeys, and humans staring directly at the camera lens. Including sound will heighten the simulation of real social events, thereby increasing the likelihood that we will observe the EEG asymmetry to, say, threats from an aggressive male Rhesus on video.

#### 2) Use of a human intruder paradigm

This stimulus is known in general to be affectively negative from the perspective of monkeys. This is especially true for the condition in which a human places his/her face 27 inches from the monkey's face, maintaining direct stare. In the event that dynamic stimuli turn out not to be suitable simulations, the human intruder should generate the expected EEG asymmetry, and provide us with the required metric of the amygdala's role in generating this asymmetry.

#### 3) Use of a fear conditioning paradigm

Fear conditioning reliably induces a state of anticipatory fear. Thus, using a conditioned fear stimulus should generate the required EEG asymmetry associated with negative affect in response to the conditioned stimulus. Once we can reliably induce this asymmetry, we will be able to determine the amygdala's role in producing this asymmetry.

#### c. Potential Adverse Effects

The only adverse effects will be due to temporary stresses, anxiety, etc., produced by using stimuli designed to be affectively negative. We will be using liquid reward intermittently and other positive reinforcement (e.g., marshmallows) between experiments, in order to bring any induced affective states back down to baseline.

Use of an air puff under fear conditioning is known to cause no damage to any part of the face.

We will work closely with CNPRC staff to monitor any indications that our subjects are experiencing long-term increased discomfort. CNPRC staff will take appropriate actions if this is the case, including use of analgesics and antibiotics.

d. Signature of Principal Investigator: \_\_\_\_\_

Date: \_\_\_\_\_