

PROTOCOL FOR ANIMAL USE AND CAREEmail to: campusvet@ucdavis.edu**CNPRC**

EH&S USE ONLY

**PROTOCOL: 10187
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 Monkeys	26	CRPRC colony

Project Title	Neurobiology of Social Perception in the Nonhuman Primate		
Overnight housing location::	CRPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> 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.

Humans and monkeys use a variety of gestures, such as facial expressions, in the service of social perception and social communication and in establishing a hierarchical structure. These studies will begin to evaluate which brain regions are essential for mediating social perception and the production of appropriate species-specific behaviors.

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.

None

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 type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input 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:	CRPRC, UCD startup funds, NIMH	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8636

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 pcillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

The goal of these studies is to determine the role of a brain region called the amygdala in the mediation of social perception and mobilization of appropriate species-specific behaviors. To accomplish this, mature male Rhesus monkeys have received bilateral amygdala lesions using the selective neurotoxin, ibotenic acid as approved and carried out under the original and approved protocol 8636. The studies in the original protocol and those proposed here, are based on the premise that the amygdala receives high level sensory information from all modalities and is responsible, in large part, for determining the species-specific relevance of ongoing sensory experiences. We hypothesize that complete bilateral lesions of the amygdala will disrupt the social perceptual system and result in inappropriate social behavior during dyadic and triadic interactions of lesioned animals with nonlesioned stimulus animals. Therefore, we will continue to collect quantitative behavioral data on the frequencies and types of social interactions lesioned monkeys make when interacting with nonlesioned stimulus animals. Furthermore, we hypothesize that the inappropriate social behavior may be related to the role the amygdala plays in regulating fear and/or anxiety in social and non-social contexts. We will investigate this assumption by presenting the subjects with nonsocial, but emotionally provocative stimuli, such as animal-like, in-animate objects and evaluating their responsiveness to these objects and potential to startle.

The amygdala also initiates the stress response through multisynaptic activation of the pituitary-adrenal system. In order to determine whether stress-responsiveness is disrupted, and, thus, potentially alters social dynamics indirectly, we will evaluate the pituitary-adrenal response to standard nonsocial stressors, restraint, and exposure to novelty.

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 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.)

All animals involved in this study have had canine teeth clipped to reduce the chance of injury during freely moving interactions. Twelve mature male Rhesus monkeys have received bilateral ibotenic acid lesions of the amygdala (N=6) or hippocampus (N=6) as proposed and approved in protocol 8636. In this protocol, these 12 animals, along with 6 controls and 8 non-operated stimulus controls were approved to undergo various behavioral testing paradigms to evaluate the normality of nonsocial and social interactions during novel and familiar dyads and tetrads. The ibotenic acid treated animals have already undergone the following procedures as proposed and approved in protocol 8636:

- 1) Surgery for implantation of glass marker beads at stereotaxically defined locations on the skull.
- 2) Magnetic resonance imaging analysis of the brain with skull beads to develop an individualized stereotaxic atlas for each monkey prior to selecting coordinates for ibotenic acid injection.
- 3) Injection of ibotenic acid hydrate (10% solution in 0.1M phosphate buffer) into either the left and right amygdala or left and right hippocampus. The latter controls for specificity in the alteration of social function of the amygdala lesions.
- 4) Minimum of two weeks of recovery.
- 5) Post-operative MRI's to evaluate lesion.
- 6) Initiation of behavioral testing with sham-operated and non-operated stimulus control animals.

The following research designs are a combination of research paradigms proposed in the original protocol, 8636, but have not yet been conducted in their entirety and new procedures aimed at answering changing research questions.

Assessments of social perception and behavior will be made in three situations: 1) **Videotape exposures.** Each animal will be placed in an individual cage and will briefly watch (approx. 10 min.) segments of unfamiliar animals displaying affective social signals (e.g. threats, fear grimaces); 2) **Dyadic social testing.** To date, each subject, including 6 amygdala, 6 hippocampal lesioned, and 6 control animals has interacted with each of the 8 non-operated stimulus control animals under the first of the following two conditions. In the first condition, a stimulus animal and a subject animal were both released into a large enclosure (approx. 8' x 18') and allowed to interact freely. In the second dyadic test paradigm, not yet conducted, subject animals will be able to move about freely in the large enclosure and interact voluntarily with a stimulus animal, that is located in a holding cage adjoining the enclosure. Animals will have full auditory and visual contact throughout, but will only be able to touch each other through the bars of the adjoining holding cage. On an equal number of

trials, subject animals will be located in the holding cage and stimulus animals will be able to interact voluntarily with the subjects. For both conditions, trials will be twenty minutes in duration, and animals will be under continuous observation by a trained behavioral technician. 3) **Social group testing.** Subject animals will be placed together in four-member social groups for a two-hour period of free interaction each day. Two lesioned and two control animals will comprise each group. After one month of such daily group formations, subjects will be reassigned to new groups and daily group formations will continue. A third such phase of a month's duration will also be conducted. All social sessions will be two hours in duration, and animals will be under continuous observation by a trained technician. The time between testing in each condition will be variable, depending on weather conditions and personnel.

Assessments of nonsocial but emotionally relevant stimuli to assess the amygdala's role in modulating fear and anxiety. 1) **Intruder testing:** This behavioral task is two-fold and will be conducted to assess temperamental anxiety and fear in response to an unfamiliar human and potentially fearful stimuli. In the human intruder paradigm, the animal will be acclimated to and tested in an adapted Lab Care Cage (83.82cm L x 101.6cm H x 80.01cm W) in a room other than its home room. For the first 10 min of testing the animal will be alone in the cage, then, an unfamiliar human will enter the room and present his/her profile and/or directly stare at the animal. A behavioral technician will also be in the room to record the animal's behavioral response(s) to the unfamiliar intruder. Secondly, a fearful stimulus, such as a life-like snake will be paired with a preferred food item to assess the animal's latency to retrieve a favorable food item when it is paired with a fearful object. Prior to testing, each animal's preferred food items will be determined by placing five sets of seven food items (35 items total) on a plastic stimulus box and recording the order of retrieval of each food item. This second procedure will also take place in the same cage mentioned above. 2) **Potentiated Startle:** In the fear potentiated startle paradigm, an otherwise neutral stimulus, such as a light, is paired with an emotionally provocative stimulus, such as a loud noise (acoustic startle). This task is designed to assess anxiety to an acoustic stimulus in bilateral amygdala lesioned, hippocampal lesioned and controls. Animals will be tested individually in an adapted lab care cage and a trained technician will be in the room to record the animal's behavioral response(s). The intensity of the acoustic stimulus will range from 90-120dB. First, animals will go through a training phase in which each will be exposed to 10 pairings of the light stimulus (CS) with an air-pulse (US) to simply test for evidence of fear-potentiated startle. Second, testing will involve presenting each animal with 10 blocks of two stimulus types (noise alone and light-noise). This will allow us to assess an animal's potential to startle to a loud noise and is proven to be an objective indicator of emotionality and anxiety in humans as well as non-human primates. The startle reflex is sensitive to changes in cognitive and emotional states, thus providing important information in the understanding of neurodevelopmental disorders.

Assessments of pituitary-adrenal function by blood sampling will be made on two different situations. All blood samples will be 2-4cc in volume drawn by arm pull by animal health technicians. 1) **Habituation to the video presentation test cage:** control and test animals will be placed in the video test cage for 20 minutes and immediately following, will have blood samples taken to provide information about adrenal-function following exposure to this cage. This first blood draw will be compared to a final blood draw taken on day 6, after several session of habituation to the cage have occurred. These habituation sessions will require that the animals will be placed in the video presentation test cage for 10 minutes, twice a day, for 5 days. On day 6, another 20-minute cage exposure will be introduced followed by a final blood draw. Comparisons between adrenal hormone levels after the first 20 minute cage introduction and the last 20 minute cage introduction may reveal important information about changes in pituitary-adrenal function due to habituation, as well as differences in pituitary-adrenal function between subjects. 2) **Adrenal response to physical restraint:** On four occasions approximately 6 months apart, each subject animal will undergo two 2-hour sessions of physical restraint in a primate chair that has been modified to reduce trauma. One of each pair of

restraint sessions will be preceded by an injection of dexamethasone (50ug/kg, i.m.), and the other will involve five blood draws during the 2-hour session, with blood drawn at the onset of restraint and every thirty minutes thereafter during the two hours that follow. For comparison, each animal tested will also undergo an identical regimen of blood draws without restraint in their home cages. Animals will be observed continuously.

Based on pilot studies and the studies done to date, the total remaining time required for the above studies is approximately three to five years, depending on weather conditions and personnel. At the conclusion of the study, the lesioned animals *may be* perfused for anatomical evaluation of the lesions, and control and stimulus animals will be returned to the colony. However, no final decision on the outcome of these animals has been made to date. Reason being, that new and innovative technology is currently being piloted by several of our collaborators, which would eliminate the need for perfusion to verify the extent and accuracy of the ibotenic acid lesions. One pilot currently under way uses PET technology to verify ibotenic acid lesions and if conclusive and reliable, we would prefer to use PET technology with these animals over perfusion. Therefore, euthanizing these animals is not preferred and has not been proposed as an end point for the lesioned animals on this project.

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	Ibotenic acid lesion of amygdala	6	3
1	Ibotenic acid lesion of hippocampus	6	3
2	Controls	6	1
3	Stimulus animals	8	1

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

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

e) **Rationale for species and numbers:** How did you determine that 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?

Cortical areas that are involved in human social function, such as the frontal lobe, are primitive or nonexistent in experimental animals such as rodents, but are highly developed in macaque monkeys. Therefore, they are excellent models of human brain function. The animals included in these 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 Mann-Whitney. This estimate of group size is based on more than ten years of experience in carrying out lesion/behavior experiments by the PI, and in the field of monkey social behavior, approximately 40 years by , 24 years by , and 26 years by .

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

Building:

CRPRC

Room:

Surgical Suite

Who will be the surgeon?

The PI and trained post-doctoral researchers.

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	i.m.	Pre-surgery
Rhesus	Isoflurane	1-2%	Inhalation	During surgery, up to 12 hrs.

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)

- 1) In eight pilot animals at the Wisconsin Regional Primate Center and in the twelve animals who have undergone these procedures to date in this existing protocol, there has been no indication either of acute or chronic distress. Since the procedure causes damage to the brain, we expected changes in behavior, including changes in food preference and decreased emotional response. Animals with amygdala lesions have continued to eat and drink adequately to maintain body weight and remain hydrated. Neither the lesion procedure nor the behavioral changes are life threatening. There was no morbidity or mortality in our current or previous research employing this technique on adult rhesus monkeys.
- 2) Animals in the social conditions (dyads and social groups) may experience some stress during early group formations. Also, during

initial social sessions, there is some risk of injury.

- 3) Animals may experience mild discomfort during blood sampling procedures.
- 4) Animals may experience moderate levels of stress during the physical restraint.

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.

To minimize chance of injury addressed in item #2 above, all animals have had their canine teeth clipped prior to dyadic or group formations. In addition, a trained technician will continue to observe the animals at all times during social sessions. In the event of aggression that could lead to injury, the technician will spray the animals with a jet of water from a hose to break up the fight; testing would be immediately terminated and animals would be removed. In our experience, this method has proven 100% effective in stopping bouts of aggression. In our testing to date, there have been no cases of aggression necessitating use of water, nor any fight wounds during unconstrained dyad testing. If a wound from a fight occur, a veterinarian would be asked to evaluate both animals.

In response to item #3 above, animals adjust rapidly to the blood-drawing procedures, and so distress for this procedure is minimal. In response to items #4 above, the standard primate chair has been modified to reduce trauma to the animal during the 2-hour restraint period. Following the 2-hour restraint, the animal will have no additional manipulations for the remainder of the day.

In the event of any injuries, either from the social manipulations or blood sampling procedures, analgesics or anesthetics may be administered at the discretion of the veterinary staff.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? Yes No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

*Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that this use of animals is not **unnecessarily** duplicative of other studies.*

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit

http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu)

or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwood@ucdavis.edu)

What was the date on which you conducted this search?

7/8/02

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 social behavior, monkey, primate
PsychInfo	1966-present	Amygdala social behavior, monkey, primate
Medline	1966-present	Amygdala, fear, anxiety and emotionality

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What were your findings with respect to alternative methodologies?

The proposed methodology has produced the most precise lesion of the amygdaloid complex. The behavioral studies that have been proposed and those that have been carried out under this protocol to date, are unique and provide for the most sophisticated analyses of behavioral sequelae of amygdala lesions. There are no better methodologies available for the proposed studies to date.

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.

The previous work was a pilot study to see if amygdala lesions would result in observable behavioral changes. The answer was affirmative, and, based on those results, more refined lesion and behavioral studies have been undertaken to provide a more comprehensive body of data on the role of the amygdala in social behavior.

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

The twelve lesioned animals may be euthanized at the end of behavioral testing in order to evaluate the adequacy of the ibotenic acid lesions. The 6 control animals and the 8 stimulus animals will be returned to the colony.

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 Macaque	Sedation	Ketamine HCl	10	i.m.
Rhesus Macaque	Overdose	Sodium pentobarbital	60	i.v.

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

The surgically naïve animals will be returned to the colony.

Date: March 25, 2003
To: Animal Care and Use Administrative Advisory Committee
 c/o Office of the Campus Veterinarian
From:
RE: Amendment to Protocol #10187

This describes an amendment to Protocol 10187 that is geared toward developing a way to non-invasively immobilize the heads of Rhesus monkeys, for use in gaze tracking experiments. The goal of the work requested in this amendment is to succeed in immobilizing the head of a Rhesus monkey subject without requiring any surgical implantation of headposts, head restraint, etc. The technology we propose to use is currently in use with human patients whenever precise head positioning is required. The subjects proposed for noninvasive head immobilization are 18 mature, male rhesus monkeys who received bilateral lesions of the hippocampus, amygdala, or were sham operated controls (6 in each group), approved under Protocol 10187.

a. Proposed Changes

No additional animals are required.

No additional animals are needed as the animals proposed for testing this non-invasive head immobilizing technique are already approved under project code AMA07, Protocol #10187.

No new personnel are required.

Under this amendment, we propose using a thermoplastic mask and a water-curable head/neck support, attached to a piece of plexiglass, to achieve head immobilization. The mask and support are shown in Figures 1, 2 & 3 (<http://www.medtec.com/products/immobilization/hn/imrthermo/default.htm>).

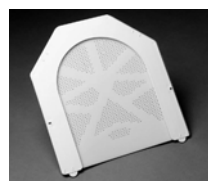


Figure 1:
Thermoplastic mask
prior to shaping

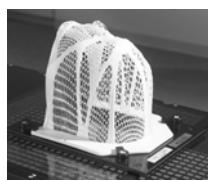


Figure 2:
Thermoplastic mask
after shaping

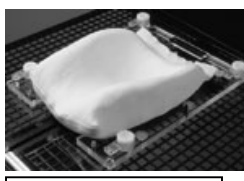


Figure 3: Water-
curable head/neck
support in
plexiglass frame

Subjects will be anesthetized using ketamine-HCl (7 mg/kg to 10 mg/kg, intramuscular) and transferred from their home cages at the California National Primate Research Center (CNPRC). There, subjects will receive additional anesthesia as needed using medetomidine (30 mcg/kg, intramuscular). Subjects' heart rate, respiration and oxygen saturation will be recorded continuously.

A thermoplastic mask will be heated for 10 minutes in a water bath set to 165 °F, until transparent. A water-curable head and neck support will be immersed in lukewarm water until soaking wet, removed and dried until only moist. It will be placed into a plexiglass frame and an anesthetized subject will be placed, face up, upon the support so that the support cradles the neck and lower cranium. Once the subject is in position, the thermoplastic mask will be removed from the water bath and, after ensuring that it is not uncomfortable to the touch, it will be sprayed with silicone spray (to prevent it sticking to the hair) and placed over the subject's face. The mask will be molded to the subject's head and face. The base of the mask will be affixed to the plexiglass frame. Both the subject's head and the mask will be held immobile for 8 minutes to 10 minutes, until the mask cools completely and hardens.

During recovery from anesthesia, CNPRC veterinarians, and laboratory investigators will monitor subjects for pain or discomfort. Atipamezol (30 mg/kg, intramuscular) may be used to reverse the effects of anesthesia. Veterinary staff at the CNPRC will provide Buprenorphine (0.01 mg/kg to 0.03 mg/kg, intramuscular) as necessary for the post-procedural relief of pain.

After a head/neck support and molded mask have been made for each subject, holes will be made for each subject's mouth and eyes. Subjects will be trained to enter a primate chair. They will be trained to sit upright, facing forward, with their head and neck back against the now upright head/neck support. They will then be trained to sit calmly while the molded mask is fixed in place.

Subjects will then be trained to look at points of light presented at different locations on a computer monitor, so that the infrared gaze tracking system can be calibrated. Subjects will be rewarded with juice for looking at the points.

Pictures of nonfacial objects and pictures of faces will then be presented, and subjects will be free to look at them in the manner in which they choose. Their gaze position will be tracked using the infrared camera. Subjects' heart

rates will be monitored during the looking behavior. Subjects will be tested for a maximum of 2 hours after the mask is in place. This duration is necessary in order to ensure enough time for both the calibration of the gaze tracking system and the experiment itself.

After the experiments (1-2 hours), the mask will be removed and the subjects will be given food reward prior to being returned to their home cages.

b. Justification

We are collaborating with a laboratory at the Salk Institute in La Jolla, CA, investigating neurological mechanisms causing Williams Syndrome. Williams Syndrome is characterized by low verbal IQ, severe visuospatial deficits and increased socially affiliative behavior, including increased eye contact. Rhesus monkeys who lack an amygdala in their brains also demonstrate increased affiliative behavior. We would like to compare how people with Williams Syndrome look at pictures of human faces with how Rhesus monkeys who lack an amygdala in their brains look at pictures of Rhesus faces. The subjects under this protocol have all already received either sham lesions, lesions to both hippocampal brain areas or lesions to both amygdaloid brain areas (6 in each group), and their behaviors have been studied extensively. They are a rich behavioral resource that could help us understand what changes in the brain take place in Williams Syndrome.

In order to study how our Rhesus subjects look at pictures, we need to aim an infrared camera at their eyes while keeping their heads still. Currently, all commercially available head immobilization technology for Rhesus monkey behavioral research requires major surgery for implementation. We plan to use the technology described here to remove any need for surgery. This, we hope, will decrease any discomfort for our subjects.

c. Potential Adverse Effects

The main risk to the subjects, aside from standard risks of anesthesia, involves the training period. During this period, subjects may experience temporary confusion, stress, etc. We will rely on using positive food rewards with our subjects (e.g., grapes, marshmallows, peanuts) in order to decrease stress and increase learning.

There are no adverse effects of the infrared camera. The infrared system is a noninvasive system for measuring eye movements, and avoids the use of a surgically implanted scleral search coil to monitor eye movements. Our infrared system (www.a-s-l.com) is identical to many in use by laboratories that work with human subjects (e.g., MRI centers, psychophysics labs, etc.).

Here, we address key questions regarding the potential adverse effects of using the individual masks.

One question is whether the masks will be too confining, so that the subjects would become too hot inside the masks. In answer to this important consideration, please note that the masks are essentially a plastic net (see especially Figure 2), so that there are numerous holes in the mask for air to circulate against the skin of the face, thereby allowing natural cooling of the skin. Additionally, we will be making large holes in the masks for the eyes and mouth, so that even more of the skin will be exposed than is typically the case when the masks are used with humans.

A second question is the maximum duration that a subject will be inside the mask. Awake subjects will be inside their masks for a maximum of 2 hours. In the case of human subjects, the maximum duration is generally 20-30 minutes, according to the supplier, Med-Tech. There is no known case of contact sores developing in human subjects from use of the thermoplastic masks, according to the supplier. Any development of contact sores will be monitored closely, and veterinary staff at the CNPRC will provide Buprenorphine (0.01 mg/kg to 0.03 mg/kg, intramuscular) as necessary for the post-procedural relief of pain. They will also monitor for the need for antibiotic treatment and supervise this treatment if it is necessary. If contact sores are determined by the veterinary staff to be a major problem, we will discontinue the use of the masks and identify another alternative for head immobilization. Once we have identified such an alternative, we will submit another amendment proposal to the IACUC.

d. Signature of Principal Investigator: _____

Date: _____

Date: April 7, 2003
To: Animal Care and Use Administrative Advisory Committee
c/o Office of the Campus Veterinarian
From:
RE: Amendment to Protocol #10187

This describes an amendment to Protocol 10187 that requests 2 additional Rhesus monkey subjects for Protocol 10187. This request is geared toward developing a way to non-invasively immobilize the heads of Rhesus monkeys, for use in gaze tracking experiments. The goal of working with the animals requested in this amendment is to succeed in immobilizing the head of a Rhesus monkey subject without requiring any surgical implantation of headposts, head restraint, etc. The technology has been described in a previous amendment.

a. Proposed Changes to 10187

Two additional male Rhesus monkeys, between the ages of 5 and 10 years of age, are requested. These two animals will **NOT** undergo structural MRIs, undergo surgery, or receive brain lesions. They will only be included in piloting the proposed head immobilization methodology and in its use with the noninvasive gaze tracking system. They will need to be anesthetized, undergo chair training, undergo head immobilization training and undergo visual fixation training (i.e., they will be trained to look at a small dot displayed on a computer screen).

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

b. Justification

We are collaborating with a laboratory at the Salk Institute in La Jolla, CA, investigating neurological mechanisms causing Williams Syndrome. Williams Syndrome is characterized by low verbal IQ, severe visuospatial deficits and increased socially affiliative behavior, including increased eye contact. Rhesus monkeys who lack an amygdala in their brains also demonstrate increased affiliative behavior. We would like to compare how people with Williams Syndrome look at pictures of human faces with how Rhesus monkeys who lack an amygdala in their brains look at pictures of Rhesus faces. The subjects under this protocol have all already received either sham lesions, lesions to both hippocampal brain areas or lesions to both amygdaloid brain areas (6 in each group), and their behaviors have been studied extensively. They are a rich behavioral resource that could help us understand what changes in the brain take place in Williams Syndrome.

In order to study how our Rhesus subjects look at pictures, we need to aim an infrared camera at their eyes while keeping their heads still. Currently, all commercially available head immobilization technology for Rhesus monkey behavioral research requires major surgery for implementation. We plan to work with the two requested additional monkeys to test the technology described in a previous amendment, in order to remove any need for surgery. This, we hope, will decrease any discomfort for our subjects.

The additional 2 subjects requested are required to ensure that all potential adverse effects of this new methodology will be identified and mitigated prior to using the proposed method with the subjects already approved for this protocol. The subjects that have already been approved for the protocol are very important to the scientific questions being addressed by the study as a whole. They have undergone key surgeries and thousands of hours of behavioral observations have been recorded for them. Thus, it is desirable not to risk their current status while piloting new methods, even ones that are as noninvasive as those described here.

c. Potential Adverse Effects

The main risk to the subjects, aside from standard risks of anesthesia, involves the period of training to the individual mask. During this period, subjects may experience temporary confusion, stress, etc. We will rely on using positive food rewards with our subjects (e.g., grapes, marshmallows, peanuts) in order to decrease stress and increase learning.

There are no adverse effects of the infrared camera. The infrared system is a noninvasive system for measuring eye movements, and avoids the use of a surgically implanted scleral search coil to monitor eye movements. Our infrared system (www.a-s-l.com) is identical to many in use by laboratories that work with human subjects (e.g., MRI centers, psychophysics labs, etc.).

Here, we address key questions regarding the potential adverse effects of using the individual masks.

One question is whether the masks will be too confining, so that the subjects would become too hot inside the masks. In answer to this important consideration, please note that the masks are essentially a plastic net (see especially Figure

2), so that there are numerous holes in the mask for air to circulate against the skin of the face, thereby allowing natural cooling of the skin. Additionally, we will be making large holes in the masks for the eyes and mouth, so that even more of the skin will be exposed than is typically the case when the masks are used with humans.

A second question is the maximum duration that a subject will be inside the mask. Awake subjects will be inside their masks for a maximum of 2 hours. In the case of human subjects, the maximum duration is generally 20-30 minutes, according to the supplier, Med-Tech. There is no known case of contact sores developing in human subjects from use of the thermoplastic masks, according to the supplier. Any development of contact sores will be monitored closely, and veterinary staff at the CNPRC will provide Buprenorphine (0.01 mg/kg to 0.03 mg/kg, intramuscular) as necessary for the post-procedural relief of pain. They will also monitor for the need for antibiotic treatment and supervise this treatment if it is necessary. If contact sores are determined by the veterinary staff to be a major problem, we will discontinue the use of the masks and identify another alternative for head immobilization. Once we have identified such an alternative, we will submit another amendment proposal to the IACUC.

d. Signature of Principal Investigator: _____

Date: _____

Date: Wed, 9 Apr 2003 10:27:31 -0700 (PDT)
From
To:
cc:
Subject: Re: Fwd: (10187) amendment

Dear,

Should I submit a revised proposed amendment, or can I reply to this question in this email? Regarding whether the subjects will be able to breathe normally during the molding of the mask, it's an important concern. Human subjects have no difficulty in breathing normally during the molding of the mask, according to representatives at MED-TECH, the makers of this technology. So we don't anticipate any difficulty in breathing for our subjects during the molding procedure, or afterwards. However, animal care and veterinary staff at CNPRC will be monitoring the breathing, heart rate and percent oxygen saturation during the molding procedure. If there is evidence that the subject is having major difficulty breathing or there is evidence that the blood oxygen levels are falling, then larger holes will be cut into the mask during the 8-10 minutes of molding or prior to molding. If this does not alleviate any problems that might arise with breathing, we will discontinue proceeding with this technology and investigate other methods. Let me know if this is helpful, or if more clarification is needed.

Best regards,

On Wed, 9 Apr 2003, wrote:

> Hi,

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> Please provide clarification about the animal's ability to breathe during
> the molding of the mask.

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> Thanks,

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>>Regarding the (10187) amendment: just to confirm the interpretation of the description and mask pictures - during the 8-10 minutes of molding/hardening the mask on the anesthetized monkey, will the animal be able to breath normally through the mask mesh?

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>>Committee members comments to your proposal: "if this replaces the need for head posts is such research, it will be a BIG step forward in reducing the stress and trauma of such research!"

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CALIFORNIA REGIONAL PRIMATE RESEARCH CENTER
 ONE SHIELDS AVENUE
 DAVIS, CALIFORNIA 95616-8542
 TEL (530) 752-0447
 FAX (530) 752-2880

Date: April 14, 2003
To: Animal Care and Use Administrative Advisory Committee
 c/o Office of the Campus Veterinarian
From:
RE: Amendment to Protocol #10187

This is an amendment to Protocol 10187 designed to secure approval on a pilot project examining fear potentiated startle response in mature Rhesus monkeys. The goal of this pilot is two-fold. 1) Examine the expected startle response patterns in adult male rhesus monkeys and 2) use the fear-potentiated startle paradigm as an investigative tool for fear conditioning in the adult rhesus monkey.

a. Proposed Changes

Add four additional animals to the already approved protocol 10187, for a temporary pilot period of 4-6 weeks. No new personnel are required.
 No additional physical, chemical or biological agents.

b. Justification

The startle response is a reflex that is preserved across different species and serves as a prophylactic reaction. It is a quantifiable response with an amplitude amenable to changes in cognition and emotionality and as such, is a powerful tool in studying the neural substrates of fear learning in the non-human primate. Under normal circumstances, the reflex amplitude is: 1) proportional to stimulus intensity; 2) habituates over repeated exposure; and 3) weakened by a closely preceding non-startle eliciting stimulus, a phenomenon known as pre-pulse inhibition (PPI). The co-occurrence of an innocuous stimulus (light or tone) with an aversive event such as an air-puff delivered in proximity to the face, results in a state of conditioned fear whereby the mere presentation of the now-conditioned light elicits reactions similar to those elicited by the unconditioned stimulus. In the presence of the conditioned stimulus, startle amplitude is enhanced when triggered in this emotional state, a phenomenon referred to as fear-potentiated startle.

This task is designed to assess anxiety to an acoustic stimulus in mature, rhesus macaque monkeys. Animals will be tested individually in custom-built wooden chamber (70 x 48 x 109 cm), which contains a restraint box custom-built to hold a rhesus monkey weighing approximately 10-17 Kg. The wooden chamber is sound-attenuated and includes a small fan producing a 65dB intra-box ambient noise. High-frequency speakers are wall-mounted producing sound in the range of 5-40 kHz and are located at subject's ear level at a distance of 12 cm. The restraint box (25 x 25 x 56 cm) includes loose-fitting belts at the waist and arms level and a grid floor upon which the animal stands. This setup serves to hold the animal in a stationary position in order to record accurate startle response readings during auditory pulse deliveries. The restraint box rests upon the startle assembly. A startle response produces a movement of the restraint box that displaces the accelerometer. The amplifier converts this motion into a voltage signal that is proportional to the chamber's displacement velocity.

Phase I: Stimulus intensity-response amplitude

Startle response will be assessed as a function of five different acoustic stimuli (90, 95, 100, 115 and 120 dB) in order to examine the relationship between whole-body startle amplitude and stimulus intensity. A 55-minute session will consist of 50 stimulus presentations as 10 consecutive blocks of 5 pulse stimuli (90, 95, 100, 115 and 120 dB) at an inter-stimulus interval of 60 sec.

Phase II: Prepulse inhibition

A 40-msec 115 dB white noise startle stimulus is presented either alone or preceded by a 20-msec 80 dB white noise non-startle eliciting prepulse stimulus. Trials consist of a pulse alone and a pre-pulse alone presentation at prepulse-pulse intervals of 45, 70, 120, 520 and 1020 msec that are systematically varied.

Phase III: Fear-potentiated startle: Fear-potentiated startle consists of two stages. **Stage 1** involves response evaluation to the light prior to any US-light (CS) pairings. This stage serves to ensure that the animals show no initial affective reaction to the light prior to the conditioning phase. **Stage 2** is the training stage that involves exposure to pairings of the unconditioned stimulus (air puff) and the conditioned stimulus (light). **Stage 3** is the testing phase and involves a comparison between the startle response in the presence of the conditioned stimulus (CS+/pulse trials) and the startle response in the absence of the CS (no CS/pulse trials).

A trained technician will be in the room to record the animal's behavioral response(s) as well as to observe the animal at all times, during all phases, via a small infrared camera mounted in the chamber that will project an image of the animal during testing to a television in test room. All training/testing will take place in the third experimental room within room 3033. Subjects will go through standard pole and collar handling procedures following which they will be trained to enter the restraint box used in the experiment. Each subject's collar will be fitted in a groove at the upper part of the box and secured with a pin. Internal loose-fitting waist and arm belts serve to stabilize the animal in a standing position and once the animal is secured the box will be bolted to the platform assembly inside the chamber. Startle amplitude will be measured in three separate paradigms where four animals will experience each phase in the same sequence: 1) stimulus intensity-response amplitude; 2) prepulse inhibition; 3) fear-potentiated startle.

c. Potential Adverse Effects.

The only potential adverse effects would be associated with the initial stages of pole and collar training which will be conducted under standard operating procedures employed at CPRC under the guidance and direction of Centralized Services. However, these stresses are expected to be minimal as the CPRC are experts at chair training and practice minimizing or eliminating an associated stress. Additional stress is expected to be associated with the acoustic noise and the air puff. However, these stresses are also minimal as the presentations are limited in duration and frequency and are highly innocuous stimuli.

