

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

EH&S USE ONLY
PROTOCOL # 9474
EXPIRES: _____

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

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

Species (common names):	Number:	Source:
Rhesus macaques	52	CRPRC

Project Title	Neurobiology of Social Perception in the Nonhuman Neonatal Primate		
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.

These studies will begin to evaluate which brain regions are essential for the developmental and mediation of social perception and the production of appropriate species-specific behaviors used in social communication within species and in establishing hierarchical social structures.

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.

Socially 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 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:	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 pctillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

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

The goal of these studies is to determine the role of a brain region called the amygdala in the development and mediation of social perception and mobilization of appropriate species-specific behaviors. To accomplish this, neonatal male and female rhesus monkeys will be subjected to bilateral amygdalotomies using the selective neurotoxin, ibotenic acid. These studies 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 social interactions of lesioned and nonlesioned animals. We hypothesize that lesions of the amygdala will disrupt some cognitive tasks thought to be mediated by the amygdala. Quantitative behavioral and cognitive data will be obtained on the amounts and types of social interactions and cognitive deficits lesioned monkeys will show as compared to nonlesioned monkeys.

The amygdala also initiates the stress response through multisynaptic activations of the pituitary-adrenal system. In order to determine whether stress-responsiveness is disrupted and, could potentially indirectly alter social dynamics, we will evaluate the pituitary-adrenal response to standard nonsocial stressors, mother-infant separation, 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.)

Twenty-four female rhesus macaques will be selected and timed-mated to conceive in four cohorts of six with no more than 10 days between expected due dates. Ultrasounds will be given to females at or near 80 days gestation to determine early differences in sex ratios per cohort (pregnant females will be moved between cohorts in severe cases of unbalanced sex ratios). Neonates born into each cohort will be randomly assigned to one of three treatment conditions; bilateral ibotenic acid lesions of the amygdala (N=8), the hippocampus (N=8), or sham operated controls (N=8). Each cohort will include six females and six neonates. Two weeks following the birth of each neonate, surgeries will be conducted. All neonates will be returned to and raised by their mothers for the first six months of life. All neonates will undergo behavioral testing to evaluate normality of social interactions with their own mothers, with other mothers, and with familiar and unfamiliar lesioned and control infant conspecifics.

During the first two weeks of the infants' life, the mother will be anesthetized approximately 3 times and the baby removed, its head shaved and daubed with substances common to surgery (e.g. betadine and ethyl alcohol). This is done in an attempt to habituate the mother to the novelty of having its infant removed prior to and returned following the surgical procedure. We have conducted this procedure with other neonatal animals that have undergone neurosurgical procedures for neuroanatomical and behavioral studies and this has led to a high success rate of mothers accepting their returning infants. At two weeks of age, experimental animals will undergo a surgical procedure according to the following protocol. 1) Magnetic resonance imaging (MRI) analysis of the brain will be performed in the morning just prior to neurosurgery to determine the exact individual coordinates for ibotenic acid injections. Infants will be awake when transported from CRPRC to Sacramento for MRI analysis and monitored by a trained technician during the move, additionally a catheter will be placed in the saphenous vessel prior to travel. Once at the imaging center infants will be anesthetized with ketamine (10mg/kg), and Metatomadine (20mcg/kg) a tracheal tube will be inserted by the attending veterinarian, Atropine (.04mg/kg) will be given sub-cutaneously and infants will be placed in a MRI compatible stereotaxic apparatus for imaging. Following MRI, infants will be transported from the imaging center to CRPRC, prepared for surgery, and anesthetized as per CRPRC Surgery SOP. Infants will be monitored continuously by a trained surgical technician and veterinarian. 2) A midline incision will be made and craniotomies performed directly above the amygdala. 3) Electrophysiological recordings will also be performed to confirm and further define the exact coordinates for the injections. 4) Injections of ibotenic acid (10% solution in 0.1 M phosphate) will be made bilaterally in the amygdala or the hippocampus. The hippocampal lesions will control for specificity in the alteration of social function of the amygdala lesions. Reciprocally, amygdala lesions will control for specificity in the alteration of cognitive and memory function in the hippocampal lesion group. Sham-operated controls will be anesthetized under the same manner as described yet only a midline incision of the skin will be performed. 5) Immediately after surgery infants will be placed in an incubator, respiration and cognitive status will be monitored by a veterinarian continuously until the vet staff has declared the infant fit to return to the home cage with its mother. After which infants will be monitored regularly until demonstrating good eating habits and steady weight gain. To ensure adequate nutrition infants may receive supplemental formula, tang, and food. 6) A follow-up magnetic resonance imaging analysis of the brain will be done during the first two weeks following neurosurgery to verify efficacy of the lesion procedure. The transport and procedures will be the same as that described above. 7) After postsurgical recovery (the length of which will be determined by the veterinary staff) mothers and infants will be moved to permanent housing adjacent to the testing area. 8) Observational studies of mother/infant and infant/infant social behavior and cognitive testing will begin. 9) All mother/infant pairs will be housed together in an

individual home cage and will be socialized with 5 consistent mother/infant pairs (and possibly one familiar adult male, for details see header: *Social Testing: #2*) 3 hr/day, 7 days/wk. 10) Mothers and infants will be monitored throughout the study by trained technicians and the veterinary staff for any signs of physical discomfort or stress.

Assessments of social perception and behavior will be made in several situations:

Social Testing.

1) Dyadic social testing. Dyad observations will include two phases of testing, pre-weaning (0-6 months) and post-weaning (6 months on). Dyads during the first six months will take place between mother/infant pairs in their home cages and in a large, open arena, social enclosure (11'x 7'x 8'). Dyads after the first six months will take place between familiar (from the same cohort) and unfamiliar (from different cohorts) infant/infant pairs in the large, open arena, social enclosure. Each neonate including amygdala lesioned, hippocampal lesioned and controls will interact with one another at a designated time during dyad testing. Two infants will be released into the social enclosure and will be allowed to interact freely. Trials will be twenty minutes in duration, and animals will be under continuous observation by a trained behavioral technician.

2) Social group testing. Social group testing will occur during two phases, pre-weaning and post-weaning. During the pre-weaning phase, neonates within an established cohort (six familiar mother/infant pairs) will be observed during a 3-hour socialization session. Up to 6 mother/infant pairs will be released into the social enclosure and will be allowed to interact freely for up to 3 hours/day, 7 days/week. The observer will sample a 20-minute trial for each neonate in the cohort and animals will be under continuous observation by a trained behavioral technician during these trials. It should be noted that there is a secondary purpose to cohort socialization, whereby we hope to promote normal social development between established social groups. Cohorts will largely be determined by infant due dates, but other considerations such as compatibility among females will be considered. To increase the chances of successfully establishing a social cohort, females will be introduced to one another prior to giving birth and long before testing begins. Females in each cohort will be gradually introduced to one another so as to reduce the risk of violence between animals, establish a dominance ranking and assess personalities. If any of the four social groups appears incompatible one, unfamiliar male conspecific will be introduced to each social group. This male will serve to stabilize the group and increase group cohesion by mediating unwarranted disputes. Once cohorts have been established infants will remain in that cohort throughout the pre and post-weaning phases of the study. Once infants have been weaned, mothers (and males) will be removed from the social group.

Social group testing during the post-weaning phase will involve observations of 3 to 6 familiar (from the same cohort) and unfamiliar (from different cohorts) infants released into the large, open arena, social enclosure and allowed to interact freely. Each infant including amygdala lesioned, hippocampal lesioned and controls will interact with one another at a designated time point during social group testing. Trials will be 20-minutes in duration, each neonate will be observed, and animals will be under continuous observation by a trained behavioral technician during these trials.

Visual Stimuli and Touchscreen Task.

1) Videotape exposures. During the post-weaning phase infants will be placed in an individual cage and will watch a brief (approx. 10 min) segment of unfamiliar animals displaying affective social signals (e.g. threats, fear grimaces) or nonsocial behavior. Subjects will view one videotape per day for five days per week; a different tape will be used for each of the four weeks that the study lasts. Two ml. of blood will be taken per animal on four occasions during this study. These data will permit us to assess the animals' social reactions to a standardized social stimulus.

2) Still image exposure. Each mother/infant pair (pre-weaning) and/or infant only (post-weaning) will be placed in an individual cage and will be

presented neutral stimuli (e.g. landscape photos), animal photos (e.g. elephants), and unfamiliar monkey photos displaying affective social signals (e.g. threats, fear grimaces), from a touch sensitive computer monitor. Animals will be trained to touch one of two/three still images presented on the screen and will receive a food reward released from an automated pellet dispenser when the animal makes the correct choice. An incorrect choice will terminate the trial and initiate the next trial. No response will also terminate the trial and after a designated timeout period a new trial will be initiated. A maximum of 100 trials will be presented to each subject on a given day, to reduce fatigue and boredom. If normal feeding time occurs four hours before testing, the animals may be fasted so that they are not satiated by the time the testing begins. The technician will feed the animals immediately following testing. The subjects will be tested on 9 different image processing tasks in an effort to evaluate the role of the monkey amygdala in processing different visual stimuli, particularly facial expressions emitted by other monkeys. Processing tasks will include, face-object, primate-nonprimate, rhesus-primate, age, gender, familiarity, gaze direction, facial expression discrimination, and facial expression matching. These tasks will permit us to determine differences among lesion and control groups in perception and memory of socially relevant visual stimuli. In addition, testing during the pre-weaning phase will allow us to observe learning and memory differences between and across lesion and control infants trained with their mothers during pre-weaning and re-tested during post-weaning.

Exposure to Novel/Social Stimuli.

1) Responsiveness testing. Mother/infant pairs and/or infants only will be tested in their living cage or placed in an individual cage with a door for loading/unloading animals, two solid walls, and a single plexi-glass front. Two small diagonal openings are made in the plexi-glass front to allow animals to have manual and oral access to food rewards, novel objects (e.g. metal lock, plastic yo-yo) and social stimuli (e.g. stuffed bear, plastic snake). Objects are securely mounted to a platform just outside of the test cage and animals are allowed access to them for up to 60 seconds/trial, with 6 trials per day. Pilot data suggests that amygdala lesioned animals are less fearful and tend to spend more time investigating novel and socially relevant objects as compared to controls. Therefore, these data will permit us to observe latency and frequency differences between lesion and control animals as well as assess responses to these objects during different developmental periods (e.g. infants at 3, 6, and 9 months) and across lesion conditions.

2) Intruder testing. On two occasions during the pre-weaning phase and two occasions following weaning each animal will be given brief behavioral tests in their living cages. During these tests, human observers will present novel objects (e.g. metal lock) to the animals, small pieces of novel food items (e.g. M&M candies), or make eye contact with (or direct facial expressions toward) the monkeys in order to assess their behavioral and emotional reactivity.

3) Mirror testing. This task will take place in the animals living cages or in an individual cage similar to that described for responsiveness testing. Mirror testing will occur during the post-weaning phase, at designated developmental time points, (e.g 7, and 9 months). Prior to testing, animals will be acclimated to the experimental setting to reduce any distress and novelty associated with the task. This phase will require that a metallic plate (approx. 8''x 11''), which limits the animals reflection but mimics a mirror be placed on the front of the cage for 5 min/per day for 1 week. A trained technician will observe and record each animal's behavior in response to the object. Following acclimation, testing will take place daily for two weeks where infants will be presented with a one-way mirror, a plexi-glass slate, or an opaque/decorative slate (control stimuli of identical dimensions to mirror) that will be affixed to the front of the cage. The behavior (e.g. avoidance, play) and facial expressions (e.g. threat, fear grimace) exhibited in response to the mirror will be recorded by the observer during five, 1 minute trials/day. This task will be conducted in order to assess the differences between amygdala and

hippocampal lesions on behavior exhibited towards a mirror and control objects and the frequency and quality of facial expressions presented to the mirror and control objects during the task at different stages in development.

Cognitive and Memory Testing.

The goal of this series of experiments is two-fold: 1) to determine the role of the hippocampus and amygdala in the emergence of declarative memory in non-human primates, 2) to determine the residual capacities following lesions of the hippocampus and amygdala.

1) Selective food satiation/ reinforcer devaluation. The satiation procedure will take place 24 hours after the subject's last feeding. A feeding box will be attached to the subject's home cage, containing 300g of food A or B (food items will include foods that are already familiar such as peanuts, raisin, grapes, sunflowers etc. Different alimentary aromas commercially available (e.g., Mc Cormick) may be added to one particular food (e.g., sunflower seeds) in order to create different types of food. The subject will be allowed to eat the food uninterrupted for up to 60 minutes. Testing will proceed 20 minutes later. Food A and food B will then be made available in the subject's home cage for up to 20 minutes. The observer will measure several parameters: Food type chosen/ eaten latency to select food and finally total amount of food ingested. Baseline sessions will be conducted in order to assess food-preferences for each animal. Food types will be counterbalanced between animals. The amount of food and the duration of the testing will be adjusted depending on the animals' behavior. The experiment will take place in the animal's home cage, a WGTA, or an experimental arena similar to the ones described below for the memory and cognitive testing. In a variation of the experiment, monkeys will be trained to discriminate objects or locations associated with two possible food rewards from non-reinforced objects or locations. After the animals have learned that discrimination, a similar procedure as used in phase 1 will be used to evaluate if lesions of the amygdala or the hippocampus affect the learning of the association between the stimuli and the value of particular food reward. Pre-weaning testing will take place when the mothers will be anesthetized for health check-up of the infants and will not necessitate any further intervention. Post-weaning testing will include the infants only.

2) Foraging task. Foraging behavior will be used to assess memory and cognition throughout development and in animals with lesions. Testing will take place during both the pre-weaning and post-weaning phases. During pre-weaning testing, a mother/infant pair will be tested in the large social enclosure (11'x 7'x 8') or a separate testing enclosure (minimal size 7'x 7'), both animals will be simultaneously observed under different experimental foraging conditions. Objects made of washable material will be placed in the testing arena as visual cues for navigating the enclosure. Monkeys will forage for food hidden under plastic feeding bowls and the observer will record the latency to retrieve the food rewards as well as the frequency of foraging attempts. Monkeys will receive up to 5 trials per day; one trial lasting up to 15 minutes. Testing will normally take place in the morning and animals will be fed immediately after testing is complete. If motivation becomes an issue, monkeys will receive their entire daily ration of food only right after the completion of the experiment. During the pre-weaning phase infants will also be tested independent of their mothers, in which case the infants will be tested on days when mothers are anesthetized for regular health check-ups by CRPRC, thereby preventing unnecessary separation of mother/infant pairs. These trials will be probe trials to assess the effects of amygdala or hippocampal lesions on acquisition and/or retention of socially acquired/transmitted information. During post-weaning testing infants will be tested under the same experimental foraging conditions in either the large social enclosure or in an infant play cage (4 X 4') previously used in the infant pilot study for this project.

3) Additional Cognitive Testing. It is anticipated that these infants will be studied through puberty, adolescence and well into adulthood. Other cognitive and social testing will likely occur at which point this protocol will be appropriately amended.

Assessments of Pituitary-Adrenal Function.

1) Mother-Infant Temporary Separation. Infants would be tested on two occasions with at least one week intervening between test days. Mothers would be anesthetized with ketamine hydrochloride (10mg/kg), to sedate them for approximately one hour. Infants would then be removed and a blood sample (1 ml, via femoral venipuncture) would be immediately collected for later assessment of basal blood levels. Infants would promptly be returned to the home cage and mothers would either remain in the home cage (as a standard disturbance control), or mothers would be removed to a remote location for the duration of the 1-hour separation. One hour following the initial blood sample, the infants would be recaptured and a second sample collected. Mothers and infants would be returned to the home cage and to each other following the separation paradigm. All testing would be conducted between 2-4 PM during the pre-weaning phase of the research 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	Neonate ibotenic acid lesion of the amygdala	8	3
1	Neonate ibotenic acid lesion of the hippocampus	8	3
2	Neonate controls	8	2
3	Mature macaque females (neonate mothers)	24	1
4	Mature macaque males	4	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 function, such as the frontal lobe, are primitive or nonexistent in experimental animals such as rodents, but are highly developed in rhesus macaque monkeys. Therefore, they are the best models of human brain function. 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, the Mann-Witney. Moreover, maternal relationships are paramount in the development of normal primate social behavior and the number of mature female mothers must match that of the animals in the neonatal groups. This estimate of group size and the decision to raise infants with their mothers for the first six months is based on more than ten years experiences in carrying out lesion/behavior experiments by the PI, and in the field of primate social behavior, approximately 40 years by , 26 years by , and 28 years by .

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	1-2	Inhalation	During surgery, up to 12 hours
Rhesus	Fentanyl	7-10mcg/kg/hr	I.V. Infusion	During surgery, up to 12 hours

Rhesus	Metatomadine	20mcg/kg	IM	Pre-surgery
Rhesus	Atipamezol	20mcg/kg	SC	May be used post-surgery to reverse effects of Metatomadine
Rhesus	Atropine	.04mg/kg	IM	Pre-surgery

h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

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 and 5 two-stage bilateral amygdalectomies/hippocampalectomies in adult rhesus monkeys using this experimental protocol at the CRPRC. Animals with amygdala or hippocampal lesions continue to eat and drink adequately to maintain body weight and in the case whereby infants do not immediately resume proper nursing and eating habits, supplemental nutrition will be provided and infants will be continuously monitored. In the three pilot infants on whom this procedure was carried out last year at the CRPRC, there was no indication of either acute or chronic stress. There was no morbidity or mortality in the entire series of surgeries performed.

Since the procedure causes damage to the brain, we expect changes in behavior including changes in food preferences and changes in emotional responses. This has been confirmed by the previous adult and infant ibotenic acid lesion studies whereby both infants and adults demonstrate and receive fewer aggressive encounters with unfamiliar conspecifics. However, animals in the social condition might experience some stress during dyad 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?

2/14/01

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, ibotenic acid, social behavior, neonate monkey, primate
PsychInfo	1966-present	Anygdala, ibotenic acid, social behavior, neonate monkey, primate

What were your findings with respect to alternative methodologies?

The proposed methodology produces the most precise lesion of the amygdaloid complex and the hippocampus and has been successfully conducted in this lab on both neonatal and mature rhesus monkeys. The behavioral studies that have been proposed are unique and provide for the most sophisticated analyses of behavioral sequelae of amygdala and hippocampal lesions 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?

The sixteen lesioned animals and the 8 controls will be kept alive for years. The experimentally lesioned animals will be studied until adulthood and may ultimately be euthanized to evaluate the adequacy of the ibotenic acid lesions.

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	10	IM
Rhesus	Overdose	Sodium pentobarbital	60	IV

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

The 24 mature female macaque mothers will be returned to the colony after six months (at which point the 24 neonates will be weaned). The non-lesioned control animals will be returned to the colony at the end of behavioral testing.

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

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

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

<hr style="border: none; border-top: 1px solid black;"/> <p><i>Principal Investigator</i></p>	<hr style="border: none; border-top: 1px solid black;"/> <p><i>Rank / Title</i></p>	<hr style="border: none; border-top: 1px solid black;"/> <p><i>Date</i></p>
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Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

<hr style="border: none; border-top: 1px solid black;"/> <p><i>Campus Veterinarian</i></p>	<hr style="border: none; border-top: 1px solid black;"/> <p><i>Date</i></p>
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