

PROTOCOL FOR ANIMAL USE AND CARE
Handwritten forms are not accepted

EH&S USE ONLY
PROTOCOL #_10635_
EXPIRES: 06/18/04_

CNPRC

Investigator

Last Name:

First:

Middle:

email:

Department:

Phones: work / home

Contact

Last Name:

First:

Middle:

email:

Department:

work / home:

Species (common names):	Number:	Source:
rhesus monkeys	approx 600/yr	CNPRC

Project Title

Overnight housing location: Day use:

Animals will be maintained by: Vivarium Investigator

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.

3-4 month-old rhesus monkey infants and their mothers will be relocated from their living cages for a 24-hr period. Infants will be assessed for individual differences in behavioral and physiological reactivity, personality, and temperament, and mothers' temperament will be assessed in their temporary holding cages.

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.

Because animals at this age are not completely weaned, they will be provided with ad lib fresh fruit and biscuits soaked in tang.

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 checked="" type="checkbox"/> Call Investigator |
| <input checked="" type="checkbox"/> Clinician to treat | <input type="checkbox"/> Save for Investigator | <input checked="" type="checkbox"/> OK to use pesticides |
| <input type="checkbox"/> Terminate | <input checked="" 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? Yes No

Radioisotopes? Yes No

Chemical Carcinogens? Yes No

Toxic Chemicals? Yes No

Agent(s):

Agent(s):

Agent(s):

Agent(s):

Funding source:

Is the project already funded? Yes No

Previously approved? Yes 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:	<input type="text"/>	Address:	<input type="text"/>
Day phone:	<input type="text"/>		<input type="text"/>
Emergency phone:	<input type="text"/>	Email:	<input type="text"/>

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.

Two decades of psychobiological research with rhesus macaques have documented the existence of stable individual differences in the organization of behavior and physiology. These differences in biobehavioral organization arise from a number of sources, are detectable at an early age, and can persist throughout life. The goal of this project is to implement an assessment program based upon this body of research that will identify differences in biobehavioral organization, and to provide this information to colony managers to aid in decision-making in the areas of health, reproduction, and enrichment. This information will also be made available to investigators who may wish to select animals with specific, defined characteristics, or who simply wish to select a more homogeneous sample for their research projects. Our plan is to characterize all available 3-4 month-old animals born into the CRPRC colony (approximately 300 per year). Because animals born into the Center's half-acre corrals have the most naturalistic rearing experience, data from these animals will provide the norms for the measures we are proposing. Finally, we will follow the animals enrolled in our assessment program as they proceed through relocations, group formations, assignment to projects, and breeding, in order to validate our measures. This assessment program is unique in the National Primate Research Center system, and will provide quantitative information to colony managers and investigators about psychological and physiological processes that are likely to influence morbidity, mortality, and research data.

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 checked="" 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 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 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.)

Approximately 300 monkeys (3-4 months of age) per year will be tested, reflecting as many of the monkeys born into the CRPRC colony as are available to us. Monkeys will be tested in cohorts of 5-8 animals as they reach 3 months of age. Each cohort will be tested for a 24-hr period, and all testing will take place during May through October.

Three-to-four-month-old rhesus monkeys and their mothers will be relocated from their living cages (eg, half-acre corrals, corncrubs, indoor housing cages, nursery) to individual holding cages indoors. Mothers will be immobilized with ketamine (10mg/kg), and infants will be removed and taken to a testing/housing area. Mothers will remain in the holding cages. Infants will be housed individually with ad lib access to food, and

a variety of novel objects and towels in their cages. During the 24-hr period, animals will be assessed behaviorally and physiologically in a number of standardized situations to identify individual differences in biobehavioral organization:

1. Focal observations (DAY 1). Each animal will be observed unobtrusively for 5 min. in its housing cage. Principal measures will be activity, vocalization, and contact with objects, and the goal is to determine individual differences in response to the initial separation from mother and relocation to a novel setting.
2. Blood sampling (DAY 1). Animals will be physically restrained briefly and a 1-ml sample of blood will be drawn via femoral venipuncture. Blood will be assayed for numbers of CD4+ and CD8+ T-cells (the ratio of which has been shown to be trait-like and under genetic control) and cortisol, a stress-related hormone. Cortisol responses to separation and relocation have also been shown to be trait-like.
3. Responsiveness to human threat (DAY 1). Using a procedure used in our other studies (and those conducted in other laboratories), animals' responses to a human displaying a profile or full frontal face will be recorded. Responses of interest include positional, activity, and emotional behaviors. Responses to this test have been shown to be stable over time (ie, reflect stable, individual differences), and are related to frontal EEG activity, and certain personality variables.
4. Social responsiveness (DAY 1). Using a videotape playback paradigm, animals will be exposed individually to a 10-min videotape of an unfamiliar animal displaying aggressive behavior. Responses of interest include positional and social behavior, and have been related to the major personality dimensions Sociability and Confidence.
5. Recognition memory (DAY 1). Each monkey will be tested using a paired comparison task. Each animal receive 10 trials. On each trial the animal will be shown two identical stimuli (objects or pictures), followed by a brief interval, after which the familiar and a novel object is displayed. The principal measure of interest is duration of looking at the novel object. This test reflects visual recognition memory, and performance has been related to later cognitive impairment, and developmental problems.
6. Pituitary-adrenal regulation (DAY 1-2). Animals will be physically restrained briefly and a 0.5-ml sample of blood will be drawn for assay of cortisol. Each animal will then be injected with 500ug/kg of dexamethasone i.m. Dex is a synthetic glucocorticoid that can suppress endogenous cortisol output. The next morning, a 0.5-ml blood sample will be taken to determine the efficacy of Dex in suppressing cortisol. Failure to suppress cortisol in humans is associated with negative affective personality characteristics (esp. depression). Finally, each animal will be injected with 2.5 IU ACTH in order to stimulate cortisol output. Thirty minutes later, a final 0.5-ml blood sample will be drawn for assay of cortisol. All blood samples will be drawn via femoral venipuncture. The dex and ACTH stimulation tests are clinical tests used routinely to determine the integrity of the hypothalamic-pituitary-adrenal system. We have data suggesting that the response of the HPA system is traitlike - individual differences in cortisol concentrations (including responses to dex suppression) are maintained over time. Because of the interrelations of the HPA and immune system, understanding of the regulation of the HPA system may provide information on which animals are at greater risk for poor health outcomes in the colony.
7. Focal observations (DAY 2). Each animal will be observed unobtrusively for 5 min. in its housing cage. Principal measures will be activity, vocalization, and contact with objects. Comparison of the Day 2 with Day 1 focal observations will provide information on individual differences in adaptability to separation and relocation, which may be important for colony management decisions.

At approximately 10 AM, infants will be reunited with their mothers in the mothers'

holding cages. Prior to the reunion, mothers' temperament will be assessed by having a trained technician hand-present three preferred food items, and record the mothers' behavioral responses. Following the reunion, mothers and infants will be observed for approximately 1 hr. to insure that the mothers will accept the infants, and to allow an opportunity for the infants to suckle. Mother-infant pairs will then be returned to their original living cages. Observations will continue to insure that the reintroductions to the cages occur safely.

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	separation from mother, blood sampling, dexamethasone injection, ACTH injection, behavioral tests.	approx. 300 infants/year	3
1	ketamine injection, relocation to indoor housing, brief behavioral assessment prior to reunion with infant	approx. 300 mothers/year	3

Categories of invasiveness

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

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

e) Rationale for species and numbers: How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

There are no specific hypotheses tested in this study. Rather, we are proposing a colony-wide assessment battery to generate information useful for colony managers' decision making about the animals in the colony. This information will be useful to identify animals at risk for poor health (both physical and psychological) outcomes. In this sense, the proposed project is similar to other assessments conducted routinely on all animals in the colony that involve immobilization and tissue collection. Rhesus macaques were selected because this is the principal species at this Primate Center. Because we will assess virtually every animal born into the colony, we are assured of having a sufficient sample size to document the full range of variation in our measures.

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 (h) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
rhesus	ketamine	10 mg/kg	i.m.	once on mother for removal of infant

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. Animals will show distress upon separation from mother. 2. Animals may have difficulty adjusting to solid food.

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.

1. The separation period will last only for 24 hrs. In addition, a towel will be placed in the animals housing cage to provide contact comfort for the infants. The towel will be available as soon as they are placed in the cage, which will be only a few minutes after separation. 2. Continuously available highly-palatable food will be provided. Should an animal refuse to eat, it will receive subcutaneous fluids at the end of Day 1, when it receives the dexamethasone injection. Finally, we will provide a 1-hr period during reunion with mother for the infant to suckle, prior to the mother and infant being returned to their group.

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:

*Are there alternative methodologies in 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: mwwood@ucdavis.edu)

What was the date on which you conducted this search?

5/21/03

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
PubMed	mid 1960s - 2003	biobehavioral organization + monkeys; biobehavioral assessment + monkeys
PsycInfo	1872-2003	same

What were your findings with respect to alternative methodologies?

There are no alternative methodologies to studying whole animals for identifying individual differences in biobehavioral organization. No alternative assessment instrument has been published in the literature.

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?

Animals will not be euthanized.

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	overdose	pentobarbital	60 mg/kg	i.v.

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

All animals will be returned to the colony.

