PROTOCOL FOR ANIMAL USE AND CARE

CRPRC

Investigator

Last Name:
First:
Middle:
Email:
Department:
Phones: work / home

Contact

Last Name:
First:
Middle:
Email:
Department:
Phones: work / home

Species (common names):
Number:
Source:
rhesus macaque
approx. 230
California Regional Primate Research Center

Project Title:
Temperament and monoaminergic influences on social competence

Overnight housing location:
CRPRC
Day use only:

Animals will be maintained by:
[x] 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.

Yearlings will be observed in their half-acre field cages. In Year 1, 30 animals will have cerebrospinal fluid samples taken, will experience brief pharmacological challenge followed by blood sampling, and will be removed for one hour to participate in social tests. In Year 2, 48 animals will have CSF samples taken, and will experience the pharmacological challenges.

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
[x] Call Investigator
[x] Call Investigator
[x] Call Investigator

Dead Animals
[x] Save for Investigator
[x] Bag for disposal

Pest Control
[x] OK to use pesticides
[x] No Pesticides in animal area

Necropsy
Necropsy
Necropsy

Hazardous Materials (only if in the animal room):

Infectious Agents?  [ ] Yes  [x] No
Agent(s):

Radioisotopes?  [ ] Yes  [x] No
Agent(s):

Chemical Carcinogens?  [ ] Yes  [x] No
Agent(s):

Toxic Chemicals?  [ ] Yes  [x] No
Agent(s):
A major task for the young organism is acquisition of the social skills needed to become a competent adult. While there are data demonstrating that intense traumatic experiences early in life compromise later social competence, much less is known about the relationship between normal variation in social competence and possible precursors in infancy. Moreover, the brain mechanisms that promote social competence in normal individuals are also poorly understood, although data from clinical populations suggest involvement of monoamine neurotransmitter systems, particularly serotonin. The proposed research is aimed at understanding the contribution of temperament to social competence and testing the hypothesis that variation in social competence is associated with variation in monoaminergic activity in vivo. Social competence will be assessed in a large sample of one-year-old rhesus macaques living in a naturalistic setting. Yearling animals experience a major transition when their mothers give birth to their next offspring. This event provides a unique opportunity to examine the changing relationship between yearling and mother, and the greater role that peer relationships take on at this time. Using both retrospective and prospective approaches, we will explore the relationship between social functioning at one year of age (with a particular focus on conflict in social transactions) and data obtained nine months earlier focusing on infant temperament (these data are collected under protocol #9122). In addition, we will contrast animals identified as low or high in social competence on three measures of central monoamine function -- genetic polymorphisms associated with serotonin function, metabolites of monoamine neurotransmitters, and neuroendocrine response to pharmacological challenge. Results from this project will increase our understanding of brain-behavior relationships through study of large samples of individuals and a comprehensive assessment of monoamine neurotransmitter function; identify prospectively risk factors for individuals at-risk of poor social outcomes; and provide a model system for development of treatments that can enhance social competence and quality of life.

b) Procedures employed in this project:
Please check the appropriate boxes if any of these procedures will be employed in your project.

- Monoclonal Antibody Production
- Polyclonal Antibody Production
- LD 50 or ID50 studies.
- Catheters, blood collection, intubation
- Prolonged restraint, (8 hrs+)
- Fasting prior to a procedure.

** 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.)

In Year 1, we will conduct behavioral observations on all yearling animals born into the 17 half-acre field cages at CRPRC, who have younger infant siblings, and who were assessed during 2001 in our ongoing project entitled ‘Biobehavioral characterization for management and research purposes’ (approved
We anticipate that there will be approximately 180 animals that will satisfy these criteria. This set of animals is referred to as 'Group 1' below. Each animal will be observed by trained behavioral observers located outside the field cage enclosures, for a period of four weeks each. The goal of this phase of the project is to determine the natural distribution of social competence in yearling animals.

Once the distribution of social abilities is determined, we will select 15 male yearlings animals that are high in social competence, and 15 male yearlings that are low in social competence for further assessments. This set of animals is referred to as 'Group 2' below. These will include:

1. Social dyad testing. Each monkey will be removed from its field cage for a one-hour period, and will be transported to an indoor testing area. It will be placed in a long ‘tunnel’ cage (5.5m long), at the end of which will be an unfamiliar adult female in a holding cage placed adjacent to the tunnel cage. The animal will be able to approach and interact with the stimulus animal during a 10-min. trial. During the one-hour test, each subject will be exposed to two different unfamiliar adult female stimulus animals, for two 10-min. trials each. The goal of this study is to determine whether yearlings identified as low or high in social competence in their familiar field cages will show differences in social behavior in a different setting with unfamiliar stimulus animals.

2. Identification of genetic polymorphisms associated with serotonin neurotransmission. As part of routine CRPRC colony procedures, blood is collected from all animals during routine health checks and cells are stored for later use by investigators interested in genotyping animals. We will utilize this tissue resource to determine the genotypes of our animals for three genes associated with serotonin neurotransmission: the promoter region of the serotonin transporter, monoamine oxidase A, and tryptophan hydroxylase.

3. Assessment of monoamine metabolites in cerebrospinal fluid (CSF). During regularly scheduled health checks, while animals are immobilized with ketamine hydrochloride, 1 ml of CSF will be withdrawn from the cisterna magna by trained CRPRC technicians using CRPRC’s approved SOP. Samples will be frozen for later assay of the major metabolites of the monoamine neurotransmitters dopamine, norepinephrine, and serotonin. Monoamine metabolites are one measure of neurotransmitter activity.

4. Prolactin response to pharmacological challenge. A neuroendocrine challenge technique will be used to measure central monoamine neurotransmitter function. Each subject will receive 4 intramuscular injections, each separated by 7 days to allow the drug to be completely metabolized. Each subject will be captured in its field cage, and a 1.0 ml blood sample will be drawn via femoral venipuncture. The animal will be injected with the agent [haloperidol (0.1 mg/kg IM), fenfluramine (4.0 mg/kg IM), clonidine (0.02 mg/kg IM) or saline control] and released. Ninety-minutes later, the technicians will re-enter the field cage, capture the animal again, and draw a second 1.0 ml blood sample. Plasma will be assayed for concentrations of prolactin, a hormone whose secretion is affected by the three neurotransmitter systems of interest. The three drugs (haloperidol, fenfluramine, and clonidine) influence neurotransmitter function of dopamine, serotonin, and norepinephrine, respectively. The prolactin response to administration of these substances is a measure of neurotransmitter activity that provides different information about the action of these neurotransmitters than simply measuring monoamine metabolites in CSF or by assessing genetic polymorphisms. Our hypothesis in all experiments is that low and high socially competent animals will show different patterns of monoamine neurotransmitter function.

We will examine the behavioral responses made by the 30 Year 1 animals on our measures of biobehavioral organization (protocol #9122), obtained one year earlier when the animals were 3-4 months of age. This will provide a *retrospective* look at whether temperament factors are influential in the development of social competence. Using this information, we will then select, in Year 2, 48 yearling rhesus monkeys based on their biobehavioral data obtained when they were 3-4 months of age. 24 animals (12 males and 12 females) will have a biobehavioral profile predictive of low social competence, and 24 animals (12 males and 12 females) will have a biobehavioral profile predictive of high social competence. We will study the behavior of these 48 animals (referred to as ‘Group 3’ below) in their field cages in response to the birth of a sibling. In addition, we will 1) draw CSF samples, as described above, to assess concentrations of monoamine neurotransmitter metabolites, and 2) assess the prolactin response to pharmacological challenge, as described above. Thus,
this study will be a *prospective* confirmation of the relationships found in Year 1 (which used a retrospective methodology), and will extend those results to females.

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Observations on field cages – year 1</td>
<td>~180</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Year 1 sample of 30: social dyad test, CSF taps, blood samples, pharmacological</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>challenge (four i.m. injections)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Year 2 sample of 48: CSF taps, blood samples, pharmacological challenge (four</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>i.m.injections)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 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 group was the minimum number necessary to achieve sound scientific results?

We will observe 180 animals in Year 1 to determine the distribution of our variable 'social competence', in order to select 15 low and 15 high socially competent animals. To determine the distribution of a trait in a population, one must either sample the entire population or employ a strategy to sample systematically. We chose to sample the entire population because we wanted to identify every low- and high- competent animal for further study. 30 animals (all males) were selected in Year 1, and 48 (half males and half females) in Year 2 were selected based on a power analysis for extreme groups, following procedures described by Feldt (1961). This sample size should permit us to detect medium to large effect sizes with power ~ 0.80.

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 (f) below.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mulatta</td>
<td>ketamine</td>
<td>10mg/kg</td>
<td>i.m.</td>
<td>Once for routine colony physicals</td>
</tr>
<tr>
<td></td>
<td>haloperidol</td>
<td>0.1mg/kg</td>
<td>i.m.</td>
<td>Once</td>
</tr>
<tr>
<td></td>
<td>fenfluramine</td>
<td>4.0mg/kg</td>
<td>i.m.</td>
<td>Once</td>
</tr>
<tr>
<td></td>
<td>clonidine</td>
<td>0.02mg/kg</td>
<td>i.m.</td>
<td>Once</td>
</tr>
<tr>
<td></td>
<td>oxymorphone</td>
<td>0.15mg/kg</td>
<td>i.m.</td>
<td>As needed for pain</td>
</tr>
</tbody>
</table>

b) 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?  
n/a

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)

No adverse effects are anticipated. Blood sample collection and CSF taps are routine procedures at CRPRC. CSF taps might result in slight discomfort. Adverse effects of haloperidol, fenfluramine and clonidine have only been reported for higher doses administered regularly over a period of weeks. We will administer a single low dose of each agent to each subject, with a 7-day washout period between injections.

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.

Oxymorphone will be administered as need to reduce discomfort from CSF taps.

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

Is death an endpoint in your experimental procedure? [ ] Yes [x] No

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

j) Literature search for alternatives and unnecessary duplication:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but those 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? [ ] 12/01/01

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search:

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>psychinfo</td>
<td>1887 - present</td>
<td>(social competence or social skills) + (catecholamines or serotonin) + monkeys</td>
</tr>
<tr>
<td>psychinfo</td>
<td>1887 - present</td>
<td>(social competence or social skills) + temperament + monkeys</td>
</tr>
<tr>
<td>pubmed</td>
<td>mid-1960s</td>
<td>(social competence or social skills) + (catecholamines or serotonin) + monkeys</td>
</tr>
<tr>
<td>pubmed</td>
<td>mid-1960s</td>
<td>(social competence or social skills) + temperament + monkeys</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are no alternatives to using whole animals for the study of social competence.

Has this study been previously conducted? [ ] Yes [x] 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 as part of this study.

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mulatta</td>
<td>overdose</td>
<td>pentobarbital</td>
<td>60</td>
<td>i.v.</td>
</tr>
</tbody>
</table>

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

All animals will be returned to CRPRC colony.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don’t need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Occupational Health Program:
Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:
Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
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/](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.

__________________________________________  Principal Investigator

__________________________________________  Rank / Title

__________________________________________  Date

Committee Use Only Below

**Conditions necessary for Committee Approval:**

**Final Disposition of this protocol:**
- Approved
- Not Approved
- 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:

__________________________________________  Campus Veterinarian

Date