PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

Investigator

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

Contact

Species (common names): ____________________________  Number: ____________________________  Source: ____________________________
Rhesus Monkey  10  CRPRC

Project Title

TEMPORARY HEARING LOSS EFFECTS ON THE CENTRAL AUDITORY SYSTEM

Overnight housing location: CRPRC  Day use only: ____________________________
Animals will be maintained by: [X] Vivarium  [ ] 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.

Animals of different ages will be anesthetized and then perfused through the heart with saline followed by fixative. Their brains will then be removed for histological processing.

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
[ ] Call Investigator  [ ] Call Investigator  [ ] Call Investigator
[ ] Clinician to treat  [ ] Save for Investigator  [ ] OK to use pesticides
[ ] Terminate  [ ] Bag for disposal  [ ] No Pesticides in animal area
[ ] 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): ____________________________
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.

Critical periods have been clearly shown in the development of the visual and somatosensory regions of the central nervous system, particularly in the cerebral cortex. In the auditory system, there is clear evidence for critical periods in the development of language acquisition in humans, as well as evidence implicating abnormal auditory cortical development in a variety of disorders including learning disabilities, schizophrenia, and autism. However, there is currently no evidence available regarding the development of the auditory central nervous system in the primate. We will test the hypothesis that there are anatomically definable critical periods in the development of the central auditory nervous system in the primate.

These experiments constitute the critical first step of this project by defining the developmental stages in the central auditory nervous system in a primate model. We expect to find that the degree in which different anatomical markers, such as calbindin, paralbumin, glutamate decarboxylase, and acetylcholine esterase immunoreactivity, endogenous cytochrome oxidase activity, and the expression of neurotransmitter receptors (GABA(A) and NMDA) will be differentiated between the newborn and the adult animal. Further, the transition from the newborn to the adult state will be defined by comparing these anatomical data across a range of age groups.

The results from this series of experiments is essential for defining likely critical periods during development and in guiding future electrophysiological studies to complement the anatomical differences with functional differences. These results are also necessary to most prudently design future experiments testing different manipulations of the auditory periphery in generating animal models of human disorders, such as learning disabilities.
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 **
- Food or water restriction
- Special diets; food or water treatment.
- Polyclonal Antibody Production **
- Non-recovery surgical procedures
- Induced illness, intoxication, or disease
- LD 50 or ID50 studies.
- Survival surgical procedures
- Death as an endpoint (see i below)
- catheters, blood collection, intubation
- Multiple survival surgery
- Trapping, banding or marking wild animals
- Prolonged restraint. (8 hrs+)
- Behavioral modification.
- Fasting prior to a procedure.
- Aversive conditioning.

** 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.)

The objectives of this study are to define the development of the auditory nervous system through a variety of histochemical and immuno-histochemical markers in fixed brain tissue. This research will fill a fundamental gap in our knowledge about the normal course of development in the primate auditory system. Similar experiments have been performed with relation to the visual system in the rhesus monkey, and thus the development of these two sensory systems can be compared.

Specific procedures: All procedures follow the CRPRC SOP for cardiac perfusion (attached). In essence the animals are deeply anesthetized (ketamine i.m. followed by nembutal i.m. or i.v.) unit there is no corneal reflex. The chest cavity is then opened, the heart exposed, a needle is inserted into the left ventricle and the right atrium is cut. The animal is perfused with saline followed by fixative. It is critically important that these procedures be initiated while the animal is alive in order to minimize necrosis and replace all blood with fixative. It is also important to emphasize that the animal is deeply anesthetized throughout the procedure. Any amendments to this SOP will be automatically adhered to, and any deviations from this SOP will not be conducted without prior approval. Procedures will be performed by CRPRC staff (when available) or by myself or members of my laboratory who have either been trained by myself or the CRPRC staff.

Numbers of animals: The scientific objectives of this study are dependent on accurately defining the time course of changes in the anatomical markers employed. The experiments are designed to minimize the number of animals based on previous studies in the visual system, however, it is not necessarily the case that the two sensory systems develop in parallel. For statistical reliability it will be necessary to obtain data from at least two animals, and potentially three if there is substantial individual variability. Thus, with the animals that have already been obtained, it is likely that individuals in only three or four age groups will be necessary. It will also be necessary to obtain two more adult animals in order to use identical fixation and other histological procedures as with the adults.

Since these animals will not be subjected to any experimental procedures prior to anesthesia and cardiac perfusion, many of these animals will be “medical culls”, particularly the adult animals. We are willing to take any animals that has no overt signs of neurological disorder, and have combined with other investigators at the CRPRC to maximize the data obtained from each animal. However, we have already had one case in which such an animals had severe hydrocephalus and an almost completely malformed cerebral cortex, thus making it inappropriate for our study.
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>Infants (0 – 6 months of age)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Adults</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no discomfort or stress&lt;br&gt;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.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration&lt;br&gt;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</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress&lt;br&gt;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</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold&lt;br&gt;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.</td>
</tr>
</tbody>
</table>

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?

As stated above, it is necessary to have several time points in order to define the time course of development. Based on studies in the visual system, it will be necessary to have at least four age groups to reliably define this time course. It will also be necessary to have at least two animals, and potentially three, at each age group to account for individual differences. These numbers (8 infants and 2 adults) are likely the minimum necessary to obtain reliable measures across this developmental time period. However, if it is the case that the mature form of all anatomical markers are noted very early, this number will be reduced.

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.

<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>Rhesus monkey</td>
<td>Ketamine</td>
<td>10 mg/kg</td>
<td>i.m.</td>
<td>Prior to experiment</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>Nembutal</td>
<td>40</td>
<td>im / iv</td>
<td>Prior to experiment</td>
</tr>
</tbody>
</table>

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)

These animals will be anesthetized and then immediately euthanized, so no adverse effects are expected.

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.

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

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

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

j) Literature search for alternatives and unnecessary duplication:

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

What was the date on which you conducted this search? [6/20/01]

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>
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<tbody>
<tr>
<td>PsychInfo</td>
<td>1887-present</td>
<td>Primate auditory development</td>
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<tr>
<td>Medline</td>
<td>1966-present</td>
<td>Primate auditory development</td>
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</tbody>
</table>

What were your findings with respect to alternative methodologies?

These are state of the art methods and no alternatives exist.

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?

At the beginning.

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>
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<tbody>
<tr>
<td>Rhesus monkey</td>
<td>Cardiac perfusion</td>
<td>Nembutal</td>
<td>40</td>
<td>im or iv</td>
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</table>

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

All will be euthanized.
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>
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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/). 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

** 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