**PROTOCOL FOR ANIMAL USE AND CARE**

*Handwritten forms are not accepted*

**CRPRC**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last Name:</td>
<td>Last Name:</td>
</tr>
<tr>
<td>First:</td>
<td>First:</td>
</tr>
<tr>
<td>Middle:</td>
<td>Middle:</td>
</tr>
<tr>
<td>email:</td>
<td>email:</td>
</tr>
<tr>
<td>Department:</td>
<td>Department:</td>
</tr>
<tr>
<td>Phone / Fax:</td>
<td>Phone:</td>
</tr>
<tr>
<td>After hrs. #:</td>
<td>After hrs. #:</td>
</tr>
</tbody>
</table>

**Species (common names):**  
Primate (*Macaca mulatta*)  
**Number:** 60  
**Source:** UC Davis CRPRC

**Project Title:** Plasticity of Primate Sensory Cortex

**Overnight housing location:** Primate Center  
**Day use only:** Primate Center

Animals will be maintained by:  
[ ] 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.

The somatosensory area of the primate cerebral cortex is the receiving area for innocuous and noxious stimuli received at the body surface. Its representation of the external world can be modified by sensory experience. The experiments investigate the brain connections that make this plasticity possible and the accompanying molecular changes that permit the cortex to adapt to changing inputs and to learn throughout the life of the individual.

**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

- [ ] Call Investigator
- [ ] Clinician to treat
- [ ] Terminate
- [ ] Necropsy

### Dead Animals

- [x] Call Investigator
- [ ] Save for Investigator
- [ ] Bag for disposal
- [ ] Necropsy

### Pest Control

- [ ] Call Investigator
- [x] OK to use pesticides
- [ ] No Pesticides in animal area

**Hazardous Materials (only if in the animal room):**

- Infectious Agents?  
  - [ ] Yes  
  - [x] No
- Radioisotopes?  
  - [ ] Yes  
  - [x] No
- Chemical Carcinogens?  
  - [ ] Yes  
  - [x] No
- Toxic Chemicals?  
  - [ ] Yes  
  - [x] No
Funding source: NIH NS21377
Previously approved? [x] Yes [ ] No
Previous protocol number (if any): 8129

What Veterinarian or veterinary clinic will provide care for your animals? (check one)
[ ] Lab Animal Health Clinic (2-0514)
[ ] VMTH Large Animal Field Service (2-0292)
[ x] California Primate Research Center (2-0447)
[ ] Another Veterinarian

If you checked “Another Veterinarian”, please provide:
Veterinarian: 
Address: 
Day phone: 
Emergency phone: 
Email: 

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 overall hypothesis is that activity-dependent plasticity of representational maps in the somatosensory cortex is based on the presence of divergent thalamocortical and brainstem connections and upon activity-dependent regulation of gene expression for molecules associated with the major inhibitory and excitatory neurotransmitter systems and involved in synaptic plasticity.

Our recent experiments (, 1998; , 2000) reveal that chronic deafferentation of the upper limb in monkeys is followed by slow atrophy of cells in the brainstem and thalamic relay centers of the sensory pathway leading to the perceptive centers of the cerebral cortex. This should be accompanied by withdrawal of the axons of these cells from higher centers, with the induction of plastic phenomena at the cellular and molecular level. These are proposed to be fundamental to any attempts to reverse the effects of long term spinal and peripheral nerve lesions in humans. The experiments are designed to determine how these phenomena form the underlying bases of plastic adaptation in the cerebral cortex.

There are two sets of experimental animals in each of which there are two subsets:
1. Control animals, not previously subjected to experimental procedures (20).

1a. Animals (10) on which no experimental procedures were performed, whose brains will be used for examination of normal patterns of expression of molecules involved in plasticity of brain connections.

1b. Animals (10) in which anatomical tracers will be injected into brainstem nuclei or the thalamus, with recovery, for examination of normal patterns of fiber connections in these structures and in the cerebral cortex.

2. Animals (40) in which the cuneate fasciculus of the spinal cord has been sectioned 6 months to 3 years previously (10 already prepared, 30 new ones to be added).

2a. Lesioned animals (10) on which no additional experimental procedures will be performed and whose brains will be used for examination of patterns of gene expression.

2b. Lesioned animals (30) in which anatomical tracers will be injected into the brainstem nuclei or the thalamus at a second operation, with recovery. In the majority of these animals, the thalamus or cerebral cortex will be mapped physiologically in a terminal experiment, without recovery, prior to euthanasia.
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 **  [X] Non-recovery surgical procedures  [ ] Induced illness, intoxication, or disease

[ ] LD 50 or ID50 studies.  [X] Survival surgical procedures  [ ] Death as an endpoint (see i below)

[ ] catheters, blood collection, intubation  [X] Multiple survival surgery  [ ] Trapping, banding or marking wild animals

[X] Prolonged restraint. (8 hrs+)  [ ] Behavioral modification.  [ ]

[X] 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.)

Specific details of the experiments. There are three kinds of surgical procedure: 1. Transection of the cuneate fasciculus of the spinal cord; 2. Introduction of microelectrodes or injection pipettes into the dorsal column nuclei (DCN) of the brainstem or into the ventral posterior nucleus (VP) of the thalamus; 3. Terminal mapping involving the same approach to the thalamus, or exposure of part of the cerebral cortex.

1. Transection of the cuneate fasciculus (animals in Groups 2a and 2b). The anesthetized animal’s head is placed in a stereotaxic frame. A midline incision is made from over the skull vertex down the back of the neck. Neck extensor muscles are reflected on one side to expose the atlanto-occipital membrane. No bone is removed. The membrane is incised to expose the upper two segments of the spinal cord and the lower part of the medulla oblongata, a procedure in which I have long experience (, 1982; , 1983; 1991; , 1992; , 1998). The cuneate fasciculus is visualized on one side at the level between the first and second segments and is cut by inserting the blades of a #4 jeweler’s forceps separated by a distance of 3mm on each side of the fasciculus and to a depth of 2mm. The blades are then closed and held together for 5 minutes which cuts the nerve fibers of the fasciculus. The forceps are withdrawn, the opening in the atlanto-occipital membrane covered with a piece of Gelfoam and the wound closed in layers. This procedure customarily lasts 1-1.5 hours. The animal recovers thereafter.

2. Injection of tracers in the DCN or VP thalamus. 6 months, 1 year, 2 years or 3 years after sectioning the cuneate fasciculus, animals in Group 2b will be re-anesthetized and:

Half of them will receive a microinjection of one of the anatomical tracers, biocytin or cholera toxin inert subunit B, into the DCN ipsilateral to the transected fasciculus.

In the other half, one or other of the same tracers will be injected into the VP thalamus contralateral to the transected fasciculus, utilizing the same occipital approach but with the addition of a small (5mmx5mm) craniotomy opening in the occipital bone, the injection pipette being introduced in the horizontal plane. Introduction of a tracer filled micropipette into the DCN or thalamus will be preceded by introduction of microelectrodes for localization purposes. Micropipettes are returned to a set of stereotaxic coordinates predetermined from angled (DCN) or horizontal (VP) passes of a tungsten microelectrode used to record receptive fields of nerve cells responding to innocuous stimulation of the body surface. Once in
place, injections are made by passing ~8 \( \mu \text{A} \) DC current through a silver wire inserted into he pipette solution or by depressing the plunger of a 1-\( \mu \text{L} \) Hamilton syringe coupled to the micropipette. These experiments normally last 4-6 hours depending on the time taken for microelectrode recording. The animal recovers thereafter.

3. Terminal mapping. 3-14 days (depending on the tracer) after the injection of tracer, the animals injected subsequent to transection of the cuneate fasciculus will be re-anesthetized and a terminal mapping procedure performed on the cerebral cortex or thalamus.

Thalamic mapping: In half the animals, the VP nucleus is mapped horizontally form behind, the microelectrode entering the thalamus through the visual cortex and midbrain, via the occipital craniotomy. Tungsten microelectrodes (~5M\( \Omega \), 0.02” diameter) are introduced, the stereotaxic coordinates being recorded. Single units and multiunit clusters are recorded systematically, using conventional methods for amplification and display of signals, as the electrode advances in 100\( \mu \text{m} \) steps and receptive fields are plotted on figurine drawings. As a detailed map is acquired as the result of repeated electrode penetrations, intermittent small marking lesions are intermittently made by passing 1-2\( \mu \text{A} \) DC current through the electrode. This does not cause convulsive activity.

Cortical mapping. The method is identical to that just described except that it occurs via a 1cm x 1cm temporal craniotomy and thinner (0.005”) microelectrodes are used to reduce risk of damage. The microelectrodes are introduced at an angle into the cortex in order to run down the anterior bank of the postcentral gyrus, neurons being recorded at 100\( \mu \text{m} \) intervals as the electrode advances. An acrylic dam is built up around the craniotomy opening and filled with mineral oil to keep the cortex moist.

There is no recovery from this procedure which lasts up to 8 hours. The animals are euthanized while deeply anesthetized.

4. Control animals

   (Group 1a) Killed without experimentation
   (Group 1b) Injected in the DCN or VP thalamus but without prior transection of the cuneate fasciculus. The approaches are the same as in 2 above. No terminal mapping is performed.

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>1a</td>
<td>None</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>1b</td>
<td>Inject brainstem or thalamus with 3-14 day survival</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>2a</td>
<td>Transect cuneate fasciculus, recover 6 months to 3 years without further procedures</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>2b</td>
<td>Transect cuneate fasciculus, recover 6 months to three years, inject thalamus or brainstem at second procedure, recover 3-14 days, terminal mapping procedure</td>
<td>30* (*10 of these animals have already received a cuneate fasciculus transection in the last protocol period)</td>
<td>3</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 groups was the minimum number necessary to achieve sound scientific results?

The use of 20 animals per year is the minimum sufficient to provide an adequate experimental series sufficient to gather enough data for statistical purposes. It is commensurate with our past experience of more than 30 years in determining what is sufficient for replicable results with this sort of material.

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

**Building:** Primate Center  
**Room:** Primate Center

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><em>Macaca mulatta</em></td>
<td>Ketamine</td>
<td>10mgm/kg</td>
<td>IM</td>
<td>Once – induction of anesthesia</td>
</tr>
<tr>
<td><em>Macaca mulatta</em></td>
<td>Isoflurane</td>
<td>Inhalation</td>
<td>Continuous – surgical anesthesia</td>
<td></td>
</tr>
<tr>
<td><em>Macaca mulatta</em></td>
<td>Pentobarbital</td>
<td>60mgm/kg</td>
<td>IV</td>
<td>Once – euthanasia</td>
</tr>
<tr>
<td><em>Macaca mulatta</em></td>
<td>Atropine</td>
<td>0.2 mgm/kg</td>
<td>SC</td>
<td>Once – antimuscarinic</td>
</tr>
<tr>
<td><em>Macaca mulatta</em></td>
<td>Oxymorphone</td>
<td>0.15mgm/kg</td>
<td>IM</td>
<td>t.i.d. 2 days postop. or p.r.n. – analgesia</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?

Muscle tone, pupil size, heart rate, blood pressure, expired CO2, temperature, (electrocorticogram when recording physiologically)

Under what circumstances will incremental doses of anesthetics-analgescs be administered?

Any of the above alterations suggestive of lightening of anesthesia

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)

Postoperatively, animals will have a midline scalp incision with sutures. There is a potential for postoperative pain and distress in all animals with surgical wounds. This could potentially be exacerbated by infection of the wound. Animals with lesions of the cuneate fasciculus will experience some loss of sensation in the hand but no paralysis and no loss of pain or temperature sensation. Because pain and temperature sensations are intact, the animals do not traumatize or self mutilate their limbs. We have found that the lesioned animals show remarkably few physical signs or limitations of mobility. The lack of deficits after these spinal lesions is one of the remarkable findings of the studies to date. Because of this, we have been able to return animals to the socially intact troops of the outdoor colony after 3-5 days to permit wound healing, and will continue to do so with appropriate observation and safeguards.

After the second experiments on lesioned animals and in the control animals with injections in the brain, the same potential for wound trauma and infection exists but there should be no additional adverse effects of the surgery. Because of the invasive nature of the surgery however, these animals will not be returned to the outdoor colony but pair housed in the indoor colony for the recovery period leading up to the terminal experiment.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of postoperative analgesics or other means, explain why this is necessary.

A careful examination will be made in the postoperative recovery period for suggestions of wound breakdown or infection and appropriate treatment will be instituted. Signs of pain unassociated with wound devitalization or self-inflicted trauma include listlessness or agitation and withdrawal from stimuli and can be treated with analgesics. Complications unmanageable by routine measures will result in a decision to euthanize the animal forthwith.

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? [June 5 2001]

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>Personal (45,000 references in Reference manager database)</td>
<td>1899-2001</td>
<td>Cortex, somatic, plasticity, thalamus, pain, primates</td>
</tr>
<tr>
<td>Medline</td>
<td>1966-2001</td>
<td>Cortex, somatic, plasticity, thalamus, pain, primates</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

I have considered possible alternatives to procedures that have potential to cause pain and distress to the animals and I have determined from my perusal of the scientific and medical journals, attendance at professional meetings over the last 40 years, and from the Animal Welfare Information Center, that there are no less painful alternative procedures that would allow the same research goals to be achieved. Examination of activity-dependent phenomena that mimic those occurring in human disease or injury require the presence of an intact nervous system in which the brain is connected to the peripheral sense organs by the nerves, spinal cord and intermediate brain levels. In vitro preparations are therefore not feasible and before data on effects can be acquired, computational models are irrelevant.

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 conclusion of each experiment.

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>Macaca mulatta</td>
<td>Anesthetic overdoses</td>
<td>Ketamine and pentobarbital</td>
<td>10mgm/kg, 60mgm, /kg</td>
<td>IM IV</td>
</tr>
</tbody>
</table>

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

There will be none
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>
<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/). 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.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
</tr>
</thead>
</table>

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.

<table>
<thead>
<tr>
<th>Campus Veterinarian</th>
<th>Date</th>
</tr>
</thead>
</table>