

PROTOCOL FOR ANIMAL USE AND CARE*Handwritten forms are not accepted***CRPRC**

EH&S USE ONLY

PROTOCOL # 9595**EXPIRES: _____****Investigator**

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

Contact

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

Species (common names):**Number:****Source:**

Primate (<i>Macaca mulatta</i>)	60	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** Call Investigator Save for Investigator Bag for disposal Necropsy**Pest Control** Call Investigator OK to use pesticides No Pesticides in animal area**Hazardous Materials (only if in the animal room):**Infectious Agents? Yes NoRadioisotopes? Yes NoChemical Carcinogens? Yes NoToxic Chemicals? Yes No

Agent(s):

Agent(s):

Agent(s):

Agent(s):

Funding source:	NIH NS21377	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8129

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pcstillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

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

The 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 or 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:

- | | | |
|---|--|--|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input checked="" type="checkbox"/> Non-recovery surgical procedures | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input checked="" type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input checked="" type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

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.

Note: Volumes: In half the animals unmeasurably small amounts by iontophoresis as stated. In the other half from 0.05 to 0.5 microliters by pressure, as stated. Half the animals will receive biocytin injections (mostly those injections made by iontophoresis); the other half will receive cholera toxin inert subunit B (mostly those injections made by pressure). Number of cells required to be labeled determines tracer used, biocytin gives the fewest, cholera toxin B the most.

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 $\sim 8\mu\text{A}$ DC current through a silver wire inserted into the 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 from behind, the microelectrode entering the thalamus through the visual cortex and midbrain, via the occipital craniotomy. Tungsten microelectrodes ($\sim 5\text{M}\Omega$, 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 $1\text{cm} \times 1\text{cm}$ 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.

Group	Procedures / Drugs	Number of Animals	Category
1a	None	10	2
1b	Inject brainstem or thalamus with 3-14 day survival	10	3
2a	Transect cuneate fasciculus, recover 6 months to 3 years without further procedures	10	3
2b	Transect cuneate fasciculus, recover 6 months to three years, inject thalamus or brainstem at second procedure, recover 3-14 days, terminal mapping procedure	30* (*10 of these animals have already received a cuneate fasciculus transection in the last protocol period)	3

Categories of invasiveness

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

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

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

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: Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

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

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
<i>Macaca mulatta</i>	Ketamine	10mgm/kg	IM	Once - induction of anesthesia
<i>Macaca mulatta</i>	Isoflurane		Inhalation	Continuous - surgical anesthesia
<i>Macaca mulatta</i>	Pentobarbital	60mgm/kg	IV	Once - euthanasia
<i>Macaca mulatta</i>	Atropine	0.2 mgm/kg	SC	Once - antimuscarinic
<i>Macaca mulatta</i>	Oxymorphone	0.15mgm/kg	IM	t.i.d. 2 days postop. or p.r.n. - analgesia

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-analgesics 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 post-operative 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 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.

Database Name	Years Covered	Keywords / Search Strategy
Personal (45,000 references in Reference manager database)	1899-2001	Cortex, somatic, plasticity, thalamus, pain, primates
Medline	1966-2001	Cortex, somatic, plasticity, thalamus, pain, primates

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 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.

Species	Method	Drug	Dose (mg/kg)	route
<i>Macaca mulatta</i>	Anesthetic overdoes	Ketamine and pentobarbital	10mgm/kg 60mgm,/kg	IM IV

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

There will be none

